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THE PRESERVATION OF THE LIFE OF THE FROG'S EGG AND THE INITIATION OF DEVELOPMENT, BY INCREASE IN PERMEABILITY

J. F. McCLENDON

Contribution from the Physiological Laboratory of the University of Minnesota[1]

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If the frog's egg is removed from the body of the female and placed in water it soon dies and disintegrates. This is true whether the egg is removed from the ovary, the body cavity or the oviduct. Even though the egg were laid in the normal fashion, it would die if not stimulated by a spermatozoon or in some other manner. Bataillon[2] was able to cause unfertilized frog's eggs to segment by pricking them with a needle. But I (1) found that a much more convenient and efficient method of causing them to segment is to stimulate them with the ordinary 110-volt alternating current from carbon or platinum electrodes placed about two inches apart in the water containing the eggs, for about one second. Bataillon thinks the introduction of a foreign protein, such as is used to induce anaphylaxis, is necessary for the development of these eggs. I found the jelly in which the eggs are imbedded to be permeable to hemoglobin, and it is possible to think of protein diffusing in without the needle prick. But eggs removed so carefully that there is no possibility of foreign protein gaining access to them, segment when stimulated electrically. It is certain that no foreign protein is necessary for the beginning segmentation of this egg.

[1] The nephelometer used in this work was bought with a grant from the Research Fund of the Graduate School.

[2] Bataillon: Comptes Rendus de la Société de Biologie, 1911, lxx, 562.

though it may be necessary for its later development. In the leopard frog, Rana pipiens, this segmentation usually stops before the first cytoplasmic division is completed, although nuclear divisions may continue for several days.

The initiation of cleavage in the frog's egg by the simple prick of a needle, which I have observed in American frogs, might be interpreted as increased permeability. Since I have shown that electrical stimulation of muscle causes increased permeability (2), electrical stimulation of these eggs might be supposed to have the same effect. In order to determine this point, I made some preliminary experiments last year, but owing to difficulty in obtaining material, could offer but meagre data to prove the point (3). This year the leopard frogs bred in the laboratory, and furnished abundant material.

A large number of females of the same size, and that were clasped by males, were placed in a tank with two inches of water. As soon as a female deposited her eggs, these were carefully removed to distilled water. Two females were opened and their eggs placed in separate dishes of distilled water, one as a control and the other for electrical stimulation.

In removing the eggs, the female was washed free from sperm, the head cut off, most of the viscera removed and the blood washed out. The distended oviducts were carefully removed and the blood washed out of the vessels in their walls. A hole was made in each oviduct and it was dragged across the floor of a dry glass dish, leaving a trail of adhesive eggs. When distilled water was added the eggs remained adhering to the glass.

In order to guard against slight differences in the masses of eggs, the two oviducts of one female were emptied in separate dishes and one used as an unfertilized control and the other stimulated electrically. The batch of fertilized eggs of another female was cut in two equal parts, and one part placed in a third dish.

The eggs were washed in repeated changes of distilled water for an hour. During this time the gelatinous coverings of the eggs partially swelled. The sperm traverses the jelly and reaches the inseminated eggs in about one hour, and the eggs rotate until their black poles are turned upward. It is now time to stimulate the unfertilized eggs in one dish. Two clean platinum electrodes, about one inch apart are passed along opposite sides of each row of eggs, until all of the eggs in the dish have been stimulated. These eggs now begin to rotate, and some of them are completely rotated in one minute. If the rows of

eggs are slender so that the jelly is uniformly swollen, all of the eggs rotate at about the same time. None of the eggs in the unfertilized control rotate, no matter how much the jelly swells. This is due to the fact that the jelly sticks tight to the egg, whereas when they are stimulated, a fluid is secreted between the egg and the jelly.

The water is now poured or syphoned off of all the eggs and a measured quantity added to each dish. Ordinary distilled water was boiled and redistilled in an automatic quartz apparatus (4) and used in the experiments. At the end of certain intervals of time, the water in each dish is stirred, and 25 cc. removed for analysis of chlorides with the nephelometer. At the end of seven hours all of the water is removed from all of the eggs. An aliquot part of it is evaporated in quartz for ash. Water is added to all of the eggs and another series of determinations made.

The following table gives a sufficient number of the results to indicate their character.

NO. OF OVIDUCTS TO EACH DISH	cc. H_2O	cc. OF A $\frac{1}{100}$ NORMAL CHLORIDE SOL. IN H_2O			GRAMS ASH IN H_2O
		0–3.5 hrs.	0–7 hrs.	7–30 hrs.	0–7 hrs.
1 unfertilized	500	0.70	1.20	0.38	0.005
1 stimulated	500	1.40	2.24	1.22	0.008
1 fertilized	500	1.22	2.20	1.20	0.007
2 unfertilized	1000	1.40	2.40	1.42	0.015
2 stimulated	1000	1.90	3.60	2.40	0.024
2 fertilized	1000	1.80	4.20	2.00	0.023
4 unfertilized	2000	2.80	4.80	3.00	0.031
4 stimulated	2000	4.00	7.60	4.80	0.053
4 fertilized	2000	3.80	7.20	4.20	0.045

The first column shows the number of oviducts used in supplying each dish with eggs, and the second gives the number of cc. H_2O added. In the first 3.5 hours as much chloride diffused out of the eggs as in 23 hours, beginning with the eighth hour. The table gives the total quantity of Cl in the H_2O as calculated from analysis of a 25 cc. sample, and the result may be converted into grams by multiplying by 0.035.

In obtaining the ash, three-fourths of the H_2O was evaporated down in quartz beakers. During the evaporation the foam was skimmed off and transferred to a platinum crucible. When the volume of the water was reduced to a few cc. it was transferred to the crucible. The

ashing was done at as low a temperature as possible by admitting air into the crucible through a small metal tube while it was heated. Some chlorides may have been lost, but the results are comparative. The ash was found to contain about 60 per cent of insoluble salts and 40 per cent of soluble salts. The ash contained Na, Li, K, Ca, Mg, Cl, SO_4 and CO_3, but no phosphates were detected. The metals were detected in the flame spectrum and the spark spectrum (fulgurator). Mg was also detected by means of the displacement of the bands of alkanin. Na was also determined quantitatively as sodium uranyl acetate and as silicofluoride but the quantities were too small to be accurate. SO_4 was precipitated with BaOH, and CO_2 driven out of the dry ash with acid.

The diffusion of salts from the electrically stimulated eggs is about the same as from the fertilized eggs, but nearly double that from the unfertilized eggs. This difference was found to continue for 50 hours, when the determinations were discontinued as the unfertilized control had died.

In making prolonged experiments it is important to change the water frequently in order to keep down the bacteria, which might bind the salts or interfere with the nephelometer reading. In previous years, I have charred and extracted the samples before nephelometric determinations, but think it a bad practice and only necessary in the presence of bacteria or protein. The traces of dissolved glyco-protein from the jelly did not cause a measurable error as a great excess of $AgNO_3$ (5 drops of a saturated solution) was added to the 25 cc. sample, and the reading was taken in one minute, whereas several hours are required for perceptible reduction of silver by these solutions in the intensity of light to which they were exposed. I found it advantageous to use open tubes closed at one end with black rubber stoppers in the nephelometer, in order to avoid the reflection from the bottom of test tubes, but this was not absolutely necessary with the Richards nephelometer, because a part of the large field shows no reflections, and test tubes can be more easily cleaned.

The supposed relation between increased permeability and cleavage has been fully discussed in a previous paper (5) in which I showed that an increase in permeability of the sea urchin's egg was produced by any treatment which causes cleavage.

The fertilized or stimulated frog's egg may live a long time, whereas the unfertilized frog's egg soon dies when placed in water. The question arises whether the increased permeability saves the life of the egg.

In order to decide this we must first consider the effect of water on the egg.

The frog's egg swells when placed in water, and I found that the unfertilized egg swells more than the fertilized or stimulated egg, until the death of the former terminates the physiological comparison. In eggs of the same female, the mean diameter of 23 unfertilized eggs 30 minutes after being placed in water was 1.52 mm. whereas the mean diameter of 23 that had been stimulated by the electric current and left in water 30 minutes was 1.47 mm. After cleavage begins the estimation of the volume of the eggs becomes more difficult, and on the formation of spaces between the cells of the fertilized egg the apparent volume is much greater than the real volume of the protoplasm. But I think that one effect of fertilization is clearly the retardation of the swelling of the egg. One need only suppose that the death of the unfertilized egg is caused by rapid swelling, in order to explain the life-saving action of fertilization or stimulation. Bachman found the unfertilized salamander egg bursts in two to seven hours if placed in water but not if in salt solution. It is not so easy to determine the exact moment of death of the frog's egg.

The retardation of the swelling of the fertilized or stimulated egg is evidently due to the loss of osmotic substances, such as the salts which I have given in the above table. At the moment of increase in permeability, a concentrated solution of these substances passes from the egg and pushes out an adhering membrane which we may call the fertilization membrane. Bialaszewicz[3] claims that a measurable decrease in volume of the egg takes place at this time. We may take this decrease in volume of the egg to equal the volume of the secreted fluid, but after this perivitelline fluid has once been secreted it increases by absorption of water through the fertilization membrane. I found this membrane to be easily permeable to salts since it cannot be easily plasmolyzed (shrunken) by immersion in hypertonic salt solutions. Hence it is probable that the perivitelline fluid contains other less diffusible substances.* An analysis of the perivitelline fluid of the Amblystoma egg showed that it contained salts and organic substances but only a trace of protein. The perivitelline fluid of the egg of the giant salamander contained 0.16 per cent of dissolved substances. Backman and Runnstrom[4] suppose these substances to be chiefly secreted by the suckers that develop on the head of the frog embryo,

[3] Bialaszewicz: Bull. Acad. Sc. Cracow, Math.-Nat., Oct. 1908.

[4] Backman and Runnstrom: Pflüger's Archiv, 1912, cxliv, 313.

but this is hardly in harmony with the fact that the salamander embryos have no suckers.

The function of the perivitelline fluid seems to be to make room for rotation of the egg and extension of the embryo, by pushing out the fertilization membrane, which is chiefly effected by the osmotic pressure of the dissolved substances that it contains. The loss of salts must be more or less independent of this, since salts are lost continuously during development, and salts would not be very effective in pushing out the membrane since it is permeable to them.

This loss of salts explains the results of Backman and Runnstrom[5] who found that the osmotic pressure of the frog's egg is reduced when it is fertilized and placed in water. They attempt to explain it, however, by erroneously assuming that fertilization causes a coagulation of the proteins and that the coagulation adsorbs the salts. They seem to consider this egg as a diphasic system in which the watery phase forms the main bulk of the egg. On the contrary, I found the cytoplasm to be a 4-phase system in which the watery phase is a very small fraction of the total volume (6). The salt would have to be lost from this watery phase only, in order to reduce the osmotic pressure of the egg, and the more permeable the plasma membrane is to them the less osmotic pressure they can exert while in the egg. I found the watery phase to be 16 per cent of the whole egg and to contain 82 per cent water (7). These figures are a little too high owing to slight admixture of other phases, but after removal of these and of the dissolved proteins, the water-soluble substances in this layer formed only 1.7 per cent of the weight of the egg.

The chlorides diffusing out of the egg in seven hours would make a solution in the watery phase more concentrated than $\frac{1}{100}$ normal and the total salts diffusing out in this time would form nearly 1 per cent of the watery phase. Since the osmotic pressure of the ovarian egg corresponds to a 0.166 normal solution of NaCl and is probably lowered in the passage of the egg through the oviduct, the increased permeability and diffusion seem to be sufficient to account for the failure of the egg to die in the very hypotonic water in which it develops.

By an analysis of the ovarian eggs, I found that the chlorides were not sufficient to raise the osmotic pressure of the watery phase to equal that of frog's blood or a 0.166 normal NaCl solution, and that they were not bound during coagulation of the protein (furthermore there is

[5] Backman and Runnstrom: Biochem. Zeitsch., 1909, 390.

no evidence that fertilization causes coagulation). About 25 cc. (26.6 g.) of ripe ovarian eggs of the leopard frog were freed from lipoids, dried, powdered and boiled in 200 cc. of distilled water, slightly acidulated with acetic acid in order to coagulate the proteins, and filtered. The ash of the filtrate required 1.55 cc. of $\frac{1}{10}$ normal $AgNO_3$ to precipitate the chlorides, whereas the ash of the coagulum required only 0.2 cc., which might have been due to that part of the filtrate adhering to the coagulum. Since this egg contains 53 per cent water, about seven times as much water was used to dissolve out the chlorides as is contained in the egg, but since the egg is permeable to salts, the whole pond is available to wash out the salts when the egg is laid in natural surroundings.

The supposition that the osmotic pressure of the frog's egg falls while it is in the oviduct is supported by the fact that such a change happens to the bird's egg. Atkins[6] found that the osmotic pressure and chloride content of the egg white is much less than that of the bird's blood. The egg white corresponds roughly to the jelly of the frog's egg, both containing some glyco-protein and being secreted by the oviduct. A coat of jelly of low osmotic pressure would cause a lowering of the osmotic pressure of the egg, provided the protoplasm is at all permeable to salts or water. Bialaszewicz[7] found that the ovarial egg of the hen has an osmotic pressure nearly equal to that of the hen's blood, but that the osmotic pressure of the yolk as it descends the oviduct, steadily decreases. The yolk extracts the water from the white so that the latter becomes denser.

Backman and Runnström state that the osmotic pressure of the fertilized frog's egg is as low as that of the pond water ($\Delta = 0.045$) but they admit that some of the jelly was adhering to the eggs that were used in the cryoscopic determination. Notwithstanding the permeability of the frog's egg it continually swells after the slight shrinkage due to the exit of perivitelline fluid, as I have repeatedly observed. Bialaszewicz made careful measurements of this swelling. Backman[8] observed the same process in the eggs of the toad and the salamander (Triton). Therefore we must conclude that the osmotic pressure of the frog's egg does not fall to the extent that Backman and Runnström suppose.

[6] Atkins: Biochem. Jour., 1909, iv, 480.
[7] Bialaszewicz: Roux's Archiv, 1912, 489.
[8] Backman: Pflüger's Archiv, 1912, cxlviii, 141.

During the development of the frog's egg and tadpole the osmotic pressure rises to become that of the adult frog. This is probably brought about by the solution of the reserve proteins (8) and the formation of osmotic substances, rather than the absorption of salts from without, since the same process takes place in the hen's egg which is cut off from osmotic exchange with the environment. Since the definitive osmotic pressure is maintained fairly constant, Backman and Sundberg[9] suppose that the skin is relatively impermeable to water, from without toward the interior, and that the elastic contraction of the cell walls resist the entrance of water produced by the osmotic pressure. On the contrary the skin of the frog continually absorbs water at a rapid rate (9), and it is only the activity of the kidneys that preserves the frog from dissolution. Any effect of the elasticity of cell walls or the skin, in preventing swelling, must be very slight, since the osmotic pressure is so great (8.9 atmospheres) and the frog will burst if the action of the kidneys is eliminated. The pronephros develops before the egg hatches and the kidney function probably develops before any danger of flooding the tissues with water occurs.

Although swelling may cause the death of the unfertilized eggs, a normal degree of swelling seems necessary for development. The egg contains 46 per cent of solid (dry) materials and the viscocity of the protoplasm is very great. If the viscocity is not reduced by the absorption of water, the segmentation of the cells of the white pole is prevented and the downgrowth of the cells of the black pole retarded. Also by the action of centrifugal force, the solid materials may be packed in the white hemisphere and its cleavage prevented (10). This hard white hemisphere exists in less degree in eggs whose swelling is prevented by immersion in solutions of too high osmotic pressure. For a number of years I have repeated experiments in which the osmotic pressure of the water was raised by the addition of salts. One-tenth molecular solutions of NaCl, and isotonic solutions of chlorides and nitrates of Na, K and Li have the same effect. If the eggs are placed in these solutions immediately after they are fertilized, and allowed to remain 24 hours, the segmentation of the white pole of some of the eggs is prevented. If they remain 48 hours in the solutions a large number are so affected, and if they remain indefinitely none of them develop. The addition of Ca or Mg does not prevent this effect, so it is probably due to osmotic pressure.

[9] Sundberg: Pflüger's Archiv, 1912, cxlvi, 225.

These salts are not entirely without toxic action, for this is manifested in the swelling of serous cavities. The swelling of the pericardium is greater in lithium solutions than in isotonic solutions of Na or K. In more concentrated solutions, the individual cells separate from one another, a condition which Roux called framboisea of the embryo.

A marked contrast exists in the effects of pure salt solutions on the eggs of the frog and those of many fish. These fish eggs are normally impermeable to salts and water and the osmotic effects are never observed. The toxic effects of salts are, however, more manifest (11). These toxic effects, resulting in the swelling of serous cavities and other abnormalities, are associated with increase in permeability (12). Though a slight increase in permeability does no damage, a greater increase causes abnormalities and a still greater increase causes death. It is probable that the swelling of the pericardium of the frog's egg is produced by increase in permeability in the same way as the swelling of the pericardium of the fish egg. The difference between the two is that, whereas the fish egg is normally impermeable to salts and to water, the frog's egg is permeable to water and to a less degree to salts. Fertilization increases this permeability to the optimum degree, and pure salt solutions increase it too much.

SUMMARY

Fertilization or electric stimulation increases the permeability of the frog's egg so that Na, K, Li, Mg, Ca, Cl, SO_4 and CO_3 diffuse out at a faster rate. Probably the swelling of the pericardium, due to the action of pure salt solutions, is caused by too great increase in permeability. Notwithstanding the outward diffusion of salts, the egg absorbs water, and the addition of osmotic substances to the medium, preventing the absorption of water, prevents the segmentation of the white hemisphere, and gastrulation.

The abnormally rapid swelling of the unfertilized egg probably is the cause of its death, and thus the increase in permeability on fertilization lowers the osmotic pressure and saves its life.

The low osmotic pressure of the egg observed by Backman and Runnström was probably caused by three processes: (1) The lowering of the osmotic pressure as the egg descends the oviduct; (2) the increased permeability and loss of salts; (3) the admixture of jelly of low osmotic pressure with the eggs in taking the freezing point.

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(3) McCLENDON: Science, 1914, xl, 70.
(4) McCLENDON: Int. Zeit. f. physik.-chem. Biol., 194, i, 31.
(5) McCLENDON: Am. Jour. Physiol., 1910, xxvii, 240.1
(6) McCLENDON: Archiv f. Zellforschung, 1910, 386, fig. 1, a.
(7) McCLENDON: Roux's Archiv, 1909, xxvii, 247.
(8) McCLENDON: Am. Jour. Physiol., 1909, xxv, 195.
(9) McCLENDON: Internat. Zeitsch. f. Physik.-chem. Biol., 1914, i, 169.
(10) McCLENDON: Roux's Archiv, 1909, xxvii, 253.
(11) McCLENDON: Am. Jour. Physiol., 1912, xxix, 289.
(12) McCLENDON: Int. Zeit. f. Phys.-chem. Biol., 1914, i, 28.

THE ACTION OF ANESTHETICS IN PREVENTING INCREASE OF CELL PERMEABILITY[1]

J. F. McCLENDON

From the Physiological Laboratory of the University of Minnesota

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Höber and Lillie have attempted to associate anesthesia with permeability. In a lecture at Woods Hole in 1911, I attempted to express the relation as follows: "If stimulation consists in increase in permeability, we should expect anesthetics to prevent this change."[2]

Most of the attempts at correlating anesthesia and permeability have been indirect. Thus Höber[3] found that anesthetics prevent the action of salts in producing a demarcation current in muscle. Lillie[4] observed that anesthetics prevent the action of salt solutions in causing artificial parthenogenesis and cytolysis of Echinoderm eggs. He also observed that anesthetics prevent the outward diffusion of cell pigment produced by salts, and thinks this a direct proof that anesthetics prevent increase in permeability. Arrhenius and Bubonovic[5] observed that anesthetics retard the hemolytic action of hypotonic solutions. Höber attributes a similar observation to Traube, but I have not as yet found the reference.

It is clear, therefore, that anesthetics in the anesthetizing concentration, tend to prevent the outward diffusion of pigment from pigmented cells. It seemed to me of interest to determine whether non-pigmented substances are affected in this way, especially the salts contained in the cells. In 1911, I had observed that the Fundulus egg is impermeable to salts, but found that Mg came out of the eggs when placed in a slightly toxic solution of NaCl.[6]

[1] This research assisted by a grant from the Research Fund of the Graduate School.

[2] McClendon: Biol. Bull., 1912, xxii, 139.

[3] Höber: Pflüger's Arch., 1907, cxx, 492, 501, 508.

[4] Lillie: This Journal, 1912, xxix, 373, 1912, xxx, 1; 1913, xxxi, 255; Journ. Exper. Zool., 1914, xvi, 591.

[5] Arrhenius and Bubonovic: Meddel. K. Ventensk. Akad. Nobel institut, 1913, ii, no. 32.

[6] McClendon: This Journal, 1912, xxix, 296.

It was later found that various toxic solutions increase the permeability of the egg to salts.[7] The addition of anesthetics to these salt solutions tends to prevent this increase in permeability.[8]

In making these last experiments, Fundulus could not be obtained and the much more delicate eggs of the pike were used. It was found that practically no salts came out of these eggs when they were placed in distilled water. Since the egg contains salts, and it does not swell or burst when placed in distilled water, it must be impermeable to water as well as to salts. When placed in slightly toxic solutions of nitrates or anesthetics the chlorides diffuse out of the egg very rapidly. But when a toxic salt solution ($\frac{1}{16}$ molecular $NaNO_3$) contains an anesthetic in the proper concentration for anesthesia, the chlorides diffuse out of the egg at a slower rate than in the pure salt solution. Thus anesthetics have two effects on these eggs, at a certain concentration they produce anesthesia and at a greater concentration they are toxic. A 3 per cent solution of alcohol or a 0.5 per cent solution of ether produces anesthesia (retards development) whereas a 6 per cent solution of alcohol, or a 2 per cent solution of ether is slightly toxic. Twice as much chloride diffuses out of eggs placed in pure $NaNO_3$ solution as in the same solution containing 3 per cent alcohol. One and a half times as much chloride diffuses out of the egg in the pure $NaNO_3$ solution as in the same solution containing 0.5 per cent ether.

These experiments were extended this year, and the chlorides determined more accurately with a Richards' nephelometer.

MATERIAL

The eggs of the pike (Esox) were used. These eggs are very delicate, and are especially susceptible to changes in temperature, lack of oxygen and to the presence of various substances in the medium. The eggs were shipped from the breeding grounds in northern Minnesota to the State Fish Hatchery in Saint Paul by being spread out in thin layers on stretched muslin in refrigerator boxes. From this point they were brought to the laboratory in a refrigerator box, and those in good condition selected one by one and placed in water that was distilled, boiled and later redistilled in an automatic quartz still.[7] No other water was used in the experiments.

[7] McClendon: Internat. Zeitschr. f. Physik.-chem. Biol., 1914, i, 28.
[8] McClendon: Sci., 1904, xxxviii, 280.

METHODS

The eggs are washed in the redistilled water until the salts practically cease coming out of them, and measured in a series of graduated cylinders of the same bore. That the chlorides have all been washed out of the transparent egg shell follows from the following facts: The last wash waters contain so little chlorides that they cannot be detected with the nephelometer unless the washings are boiled down to a small volume. Tap water usually contains 100 to 10,000 times as much chlorides. The eggs are made permeable to chlorides by $\frac{1}{10}$ molecular $NaNO_3$ solution, and while they remain in this, twice as much chloride diffuses out of the eggs in six hours as in three hours. If these chlorides were held in the thin shell we would expect more to come out in the first three hours than in the second three hours. Since the shell is at all times easily permeable to salts, the source of the chlorides is not the fluid between the egg and the shell, and the small amount of shell substance is probably not the source of such large amounts of chlorides as were obtained from the eggs. I found that when the Fundulus egg is made permeable to chlorides the other salts diffuse out also, and when the frog's egg is made permeable to Cl that SO_4, CO_3, Na, K, Li, Mg and Ca diffused out also. This is probably true of the pike egg also, and enough salts could probably be obtained by diffusion to equal the entire weight of the egg shell, which is protein in composition. The most reasonable hypothesis is that the plasma membrane is practically impermeable to salts (and to water) and that it is made permeable by toxic solutions. The permeable egg may live and develop for several days, showing that the entire store of salts is not necessary for its immediate needs.

EXPERIMENTS

The same volume of eggs was placed in each of a series of Stender dishes, with 30 cc. of the solution to be tried. At the end of six hours, 25 cc. of this solution was removed with a pipette having a trap to prevent contamination with saliva. The sample was transferred to the nephelometer tube, and five drops of a saturated solution of $AgNO_3$ added and mixed. The two matched test tubes used in the nephelometer were filled up to the zero point of the scale by the 25 cc. + 5 drops.

In case any eggs died during an experiment, this experiment was thrown out. Since the material was available in great abundance,

a number of experiments were performed, of which the following are typical:

(1) 5 cc. of eggs in each dish. Chlorides expressed in arbitrary units. Ethyl alcohol expressed in per cent by volume.

Solution.....	$\frac{N}{10}$ $NaNO_3$	+1% alcohol	+2% alc.	+3% alc.	+4% alc.
Chlorides.....	100	60	50	45	60

(2) 15 cc. of eggs in each dish

Solution.....	$\frac{N}{10}$ $NaNO_3$	$\frac{N}{10}$ $NaNO_3$+2% alc.	$\frac{N}{10}$ $NaNO_3$+3% alc.
Chlorides.....	100	50	40

The duration of the experiment, six hours, was selected because this was the maximum duration that could be considered safe. Even then, some of the experiments had to be thrown out because one or two eggs died before they were completed. The $NaNO_3$ has to be pure in order to have the right degree of toxicity. The ethyl alcohol was redistilled over metallic sodium. Although the dishes were covered in all experiments, the experiments with ether were discontinued after it was found that ether has the same effect as alcohol, owing to the fact that some of the ether diffused into the air above the eggs and the concentration of that remaining in the solution was no longer known. It is probable that all anesthetics have the same action.

A word of caution to any one who wishes to make similar experiments on pike eggs: Some of my early experiments were irregular in results. I believe the reason for this is that the egg is made more permeable by increase in temperature,[9] and that different eggs are not affected by the same temperature. I did not determine the maximum temperature at which it is safe to work, but a temperature of about 8° is safe and is easily maintained in a refrigerator. Fundulus eggs may be used at room temperature in Woods Hole, and are preferable in every way. It is clear that 2 to 3 per cent by volume of ethyl alcohol partially inhibits the permeability-increasing action of a pure $NaNO_3$ solution. It is theoretically possible to entirely prevent this increase in permeability if the toxicity of the $NaNO_3$ solution is low enough. The difficulty in demonstrating this lies in the fact that the toxicity of the sodium salt does not depend on its absolute concentration, but

[9] Osterhout: Biochem. Zeitschr., 1914, lxvii, 272.

on the ratio of sodium to calcium. With a very mildly toxic solution of $NaNO_3$ the Ca diffusing out of the egg at the first increase of permeability lowers the toxicity and hence the permeability to such an extent that no permeability increase can be measured by means of the nephelometer, and electric conductivity experiments with their large and numerous sources of error would have to be substituted.

That 2–3 per cent ethyl alcohol is really the anesthetic concentration, follows from the fact that it retards the development of these eggs. The same concentration may not be correct for every species or every tissue. In general, it seems that nerve tissue requires a less concentration of an anesthetic for anesthesia than other tissues. But it is hardly justifiable to assume that this affect of anesthetics on permeability is peculiar to egg tissue. Pike embryos were found to behave the same as eggs up to the time of the development of kidney function, when the excretion of salts interferes with the method used to measure permeability. It is probable that anesthetics retard the increase in permeability of any cell by any "stimulus."

DISCUSSION

In 1910 I observed that chloroform, when added to the sea water, reduces the electric conductivity of sea urchin eggs. This experiment was not repeated, owing to lack of time and the large number of sources of error that must be guarded against in order to be sure that decreased conductivity indicates decreased permeability. Osterhout,[10] by finding a tougher material, was able to show that anesthetics decrease the permeability, at least of certain plant cells. Joel[11] found that anesthetics decrease the permeability of erythrocytes. The question arises whether anesthetics prevent increase in permeability by decreasing permeability. If a cell is absolutely impermeable its permeability cannot be decreased. The Fundulus egg is so nearly impermeable to salts that it would be extremely difficult to measure a decrease in permeability. The pike egg may be more permeable to salts but it would be no easier to measure a decrease in permeability, owing to the delicate nature of the egg and danger of non-uniformity of material. It seems probable, however, that the anesthetic and toxic substances act on the same constituent of the cell surface or plasma membrane. If the cell surface is composed of a mosaic of different constituents,

[10] Osterhout: Sci., 1913, 111.
[11] Joel: Pflüger's Arch., 1914.

and one constituent is permeable, the cell is permeable. But if one constituent is impermeable the whole cell is not necessarily impermeable, since diffusion can take place through the other constituent. If the $NaNO_3$ makes the cell permeable by acting on a protein it is difficult to see how the anesthetic could antagonize this effect by acting on a lipoid.

Stimulation and anesthesia seem to be antagonistic states. I have shown that the permeability of striated muscle is increased on stimulation[12] and that the permeability of the eggs of the sea urchin and the frog increases when they pass from the state of repose into that of activity.[13] The question arises whether this is true of other cells, such as those of glands. In order to decide this we must discuss the psycho-galvanic reflex.

If a constant electric current is sent through the body from non-polarizable electrodes and the person is given a nervous shock, as by sticking a pin in him unexpectedly, the strength of the current is momentarily increased. Leva[14] showed that the degree of this change is in direct ratio with the number of sweat glands per unit area of the skin, and therefore concluded that the sweat glands produce this phenomenon. Gildemeister[15] was able, by improvements in the electric conductivity method, to show that this is due to the increased permeability, presumably of these glands. Apparently the gland cells are stimulated by the sympathetic nerves and their permeability is increased.

It seems, therefore, that the increased permeability on stimulation is a general phenomenon, and we might expect the action of anesthetics in preventing this increase to be general, also.

Warburg[16] has shown that anesthetics retard the oxidation of oxalic acid by blood charcoal to about the same degree as they decrease the respiration of nucleated erythrocytes. I have repeated and confirmed these experiments. Tashiro and Adams[17] have shown that the nerve fiber gives out less CO_2 when it is anesthetized. Although it was shown by Warburg[18] that the respiration of sea urchin eggs is only slightly

[12] McClendon: This Journal, 1912, xxix, 302.

[13] McClendon: This Journal, 1910, xxvii, 240, and ibid, in press.

[14] Leva: Münch. Med. Wochenschr., 1913, 2386.

[15] Gildemeister: Münch. Med. Wochenschr., 1913, 2289; see also Schwartz: Zentlbl. f. Physiol., 1913, xxvii, 734.

[16] Warburg: Pflüger's arch., 1914, clv, 147.

[17] Tashiro and Adams: Int. Zeitschr. f. Physik.-chem. Biol., 1914, i, 451.

[18] Warburg: Zeitschr. f. Physiol. Chem., 1910, lxvi, 306.

reduced during anesthesia, it seems generally true that anesthetics may antagonize oxidations by cells, oxidases and some inorganic katalyzers. The question whether permeability has any relation to the oxidative processes cannot be finally settled until we know more about the mechanism of the latter. At present we can, at most, make the generalization that in the presence of O_2 cell respiration varies more or less with cell permeability so long[19] as the cell is alive. The only observation that I know of that might appear to extend this rule to a dead cell is that of Warburg.[20] He found that the oxidation in the young erythrocytes of the goose is increased by freezing and thawing provided they are closely packed. Freezing and thawing causes hemolysis and increases permeability, and the hemolyzed cells might be considered dead.

SUMMARY

Anesthetics in the concentration that retards development (2–3 per cent alcohol or 0.5 per cent ether) tends to inhibit the permeability-increasing action of a $\frac{1}{10}$ molecular solution of $NaNO_3$ on the eggs and embryos of the pike (Esox).

[19] McClendon and Mitchell: Journ. Biol. Chem., 1912, x, 459.
[20] Warburg: Zeitschr. f. Physiol. Chem., 1911, 419.

NEW HYDROGEN ELECTRODES AND RAPID METHODS OF DETERMINING HYDROGEN ION CONCENTRATIONS

J. F. McCLENDON

From the Physiological Laboratory of the University of Minnesota

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The technique of hydrogen electrodes as applied to pure chemistry is in a high state of perfection[1] but the impression is sometimes given that these electrodes require a skilled physical chemist to use them correctly. It is true that some solutions give trouble, for example, a pure KCl solution. Starting with Merck's highest purity KCl, I recrystallized it five times in fused silica dishes and dissolved it in conductivity water, but could not obtain a neutral reading with the hydrogen electrode until I poured it boiling hot into the electrode and passed a rapid stream of hydrogen through it while cooling and while making the reading. This difficulty is never experienced with solutions containing considerable amounts of carbonates, phosphates or proteins, and hence biological fluids are less difficult than some inorganic solutions. In fact, it is not necessary with biological fluids to change the hydrogen in the electrode during the reading.

The time required to determine the reaction of a fluid depends chiefly on the time required to saturate the electrode with hydrogen. Since gold absorbs comparatively little hydrogen, it becomes quickly saturated. I found No. 36 gold wire very satisfactory from this standpoint but the electrodes made of it could not be so conveniently cleaned by heating, as platinum electrodes. Drucker made electrodes of films of iridium burned on Jena glass, but the same objection holds true of them. By reducing the thickness of the platinum, the saturation time may be reduced. I found that platinum foil 0.02 mm. in thickness, coated with platinum black, requires less than two minutes for saturation, provided it is separated from the hydrogen by only a film of the solution. Since the electrodes which Michaelis designed for rapid

[1] Ostwald-Luther: Physiko-chemischer Messungen, 3d ed. 1910, Leipzig.

work require thirty minutes for saturation[2] a considerable saving of time is thus accomplished by simply using narrow strips of thin foil instead of the wire that he used.

The chief difficulty in determining the H^+ concentration of biological fluids arises from the fact that they contain dissolved gases. The dilution of the hydrogen with other gases causes an error in the direction of greater acidity. The loss of CO_2 from the solution increases its alkalinity, hence the passage of CO_2 and O_2 from the solution into the hydrogen causes two errors which tend to oppose one another, and hence the reading might happen to be correct. Höber, Hasselbalch, Michaelis and others have guarded against the error due to escape of CO_2 from the solution. The method of Michaelis depends on the use of a very small volume of H_2 in ratio to the volume of the solution, and is best adapted to rapid work.

The chief difficulty with other gases is experienced in determinations on arterial blood. The oxyhemoglobin gives out so much O_2 into the H_2 as to cause a greater error than arises from the escape of CO_2. Milroy[3] centrifuged the blood and then poured it through the air into the electrode. In order to obviate errors arising from this procedure, as well as other errors, and to shorten the time required for a determination, I designed the following electrode:

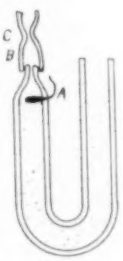

Fig. 1

This electrode consists essentially of a U-tube with one end constricted, figure 1. Next to the constriction, a strip of platinum foil, 0.02 mm. in thickness or thinner, is fused through the glass so that the plane of that portion of the foil which protrudes into the interior of the tube passes through the axis of the tube. The free end of the foil, *A*, is bent up so that when a loop of wire is passed over the constricted end of the tube electric contact will be made with the foil. A short piece of rubber tube, *B*, is attached to the constricted end of the glass tube, and closed with a small Langenbeck clip or pinch cock at *C*. The rubber tube is as short and of as small bore as practicable, and its free end is connected with a hypodermic needle. The large end of a hypodermic needle is filed down so that it may be inserted into the rubber tube. The platinum is coated with platinum black and cleaned in the usual manner.

[2] Michaelis: Die Wasserstoffionenkonzentration, 1914, Berlin.
[3] Milroy: Quart. Journ. Exper. Physiol., 1914, 141

Before filling the electrode, the needle is dried thoroughly be means of a suction pump and filled with oil. It may then be boiled for sterilization. The rubber tube is filled with a concentrated solution of hirudin in water or Ringer. The needle is inserted into the artery or vein and the Langenbeck clip removed. The U-tube is held in such a position that on'y the first few drops of blood come in contact with air, as this first blood covers and protects the rest of the blood. When the U-tube is filled, the Langenbeck clip is put on the rubber tube and the needle is removed.

The U-tube is now placed in the shield of a centrifuge, counterbalanced, and centrifuged a few minutes. It is then removed and a pipette inserted into the free end of the rubber tube. By sucking on the pipette and at the same time partially opening the Langenbeck clip, the blood corpuscles that remain in the rubber tube are removed. A tube from which pure hydrogen is flowing is instantly put in place of the pipette, without admitting any air. By cautiously opening the Langenbeck clip, hydrogen is admitted until the platinum foil is surrounded by the gas. A film of plasma adheres to the platinum and sides of the glass tube and establishes electrical connection with the blood below. After waiting two minutes for the platinum to be saturated with H_2, the U-tube is shaken so as to bring a fresh portion of the plasma in contact with the platinum, and the reading is immediately taken. Care should be taken not to shake so hard that any of the corpuscles rise high enough to liberate any oxygen into the hydrogen. In order that no time be lost in connecting this electrode with the calomel electrode, a ball of cotton cord soaked in a saturated solution of KCl is kept at hand and a piece cut off previously, with which to make the connection. The inclosure of this conducting cord in a tube is unnecessary.

Since the reading can be made in a few seconds with a proper potentiometer, the CO_2 does not have time to diffuse out of the film of plasma covering the platinum and cause an error. Since the erythrocytes have all been precipitated away from the surface layers, and the exposure is but little more than two minutes, the contamination of the hydrogen with other gases is very slight, and the error due to this unmeasurable.

This electrode has been indispensable in my work on blood, but it can be used with any biological fluid. In studying stomach or duodenal contents, the rubber tube may be connected to the smallest size stomach or duodenal tube, having a strainer (bucket) on the end that

is swallowed. The filling of the U-tube may be assisted by aspiration through a rubber tube attached to its free end, but the suction should not be sufficient to cause bubbles to appear in the fluid.

In case the readings are made at room temperature, it will be necessary to insert the U-tube in mercury in order to cool it with sufficient rapidity. If blood or any other fluid that requires to be centrifuged is used, the centrifuge shield may be filled with water so that most of the cooling takes place without loss of time.

If extreme accuracy is not required and the fluids to be investigated, contain no oxyhemoglobin, the electrode designed by Michaelis may be used, provided thin platinum foil is substituted, and a bulb is blown on the open end of the glass tube to prevent spilling while introducing the H_2. A strip of foil 0.05 mm. thick may be drawn through a small rubber stopper by means of a needle and thread, instead of sealing it in a glass stopper. This foil may be cleaned by heating, provided the stopper is first wet with distilled water, whereas a glass stopper is liable to crack.

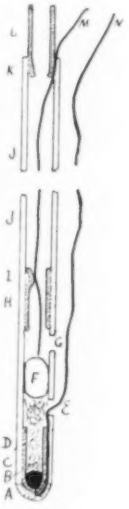

Fig. 2.

In measuring the acidity of the gastric contents, it was found possible to lower an electrode into the stomach. The apparatus designed for work on the stomach contents consists chiefly of a rubber tube 60 cm. long and 3 mm. bore, and two No. 40 silk covered copper wires, that were coated with rubber cement and dried several times (fig. 2). One wire, *M*, extends through the rubber tube, *JJ*, and the other, *N*, passes down outside of it until by entering the hole, *E*, it connects with a platinum wire that is fused into the lower end of a short piece of glass tube that is inserted into the rubber tube. The lower end of the glass tube and copper-platinum junction is covered with sealing wax, *A*. A drop of pure mercury is dropped into the lower end of the glass tube so as to connect with the platinum wire at the level of *B*. Above the mercury a little calomel washed with concentrated KCl solution, *C*, is placed, and the rest of the glass tube packed with moist KCl crystals, *D*, and the hole, *E*, stuffed with cotton soaked in KCl solution. This forms a calomel electrode, and is separated off from the remainder of the tube by a short piece of glass rod, *F*. Above *F* several holes are cut in the rubber tube at the level of *G*, and from this point a fine platinized platinum wire extends through the lumen of the tube and is held in place by fusion

to a bump on the inside of a short piece of glass tube at the level of I. This platinum wire then connects with the wire M and the junction is coated with rubber. The rubber tube is connected at K with a tube, L, leading from a hydrogen generator, and a slow stream of H_2 passes down the rubber tube and out at G, thus converting the platinum wire from H to F into a hydrogen electrode. Whereas the results are not quite as accurate as those obtained with larger electrodes, I think them sufficiently accurate for stomach contents, where there are such great individual variations. It is necessary to have a source of hydrogen of sufficient pressure to prevent the stomach contents from rising in the tube higher than H. Fresh crystals of KCl must be put in D before the apparatus is used, and the end of the tube from A to G may be immersed in saturated KCl solution so as to keep it moist until it is swallowed. A correction is made for hydrogen pressure (subtract 0.17 mv. for 1 cm. increase in pressure).

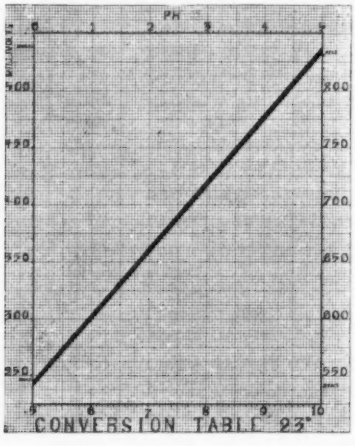

Fig. 3

The time necessary to calculate the H^+ concentration from the potentiometer reading may be saved either by using a conversion table or making a potentiometer that reads off directly the H^+ concentration. It is most convenient to express results in the form of the hydrogen ion exponent, PH. Thus 0.001 normal is 10^{-3}, or PH = 3. Figure 3 is a conversion table to be used at 23°, provided the hydrogen electrode is connected with a calomel electrode of the saturated type (containing KCl crystals). The coördinates are to be followed only to the edge of the diagonal band. Starting with the potentiometer reading in millivolts on the ordinate, the corresponding PH on the abscissa may be read.

The temperature coefficient is small and is practically zero for an H^+ concentration of 0.0001 normal (PH = 4). That is to say a 0.0001 normal solution of hydrogen ions will give a reading of 481 millivolts whether the temperature is 19°, 25° or 37°. If 23° is taken as the standard, at 19° there is a variation of +3 millivolts for PH = 0 and

-5 mv. for PH = 10. At 37° the variation is -11 mv. for PH = 0 and $+16$ mv. for PH = 10.

Since the PH of gastric juice is not far on the acid side of 4, that of urine is about 4 and that of the other biological fluids (except those containing much bile) is not far on the alkaline side of 4, a slight variation of the temperature from 23° does not make a serious error in using this conversion table or a potentiometer reading the PH directly.

None of the potentiometers on the market can be easily adapted to read the PH directly, but I made one that will serve all such purposes. It consists of 2200 cm. of No. 30 hardened German silver wire stretched over cross section paper. By means of a Weston cell, and 2 movable contacts, the current of a lead storage cell passing through a variable length of the wire is so adjusted that each centimeter has a fall of potential of one millivolt. By means of a conversion table the PH is marked on the cross section paper. We thus have a scale reading the PH instead of the millivolts. See also the description of a direct reading potentiometer in this journal.

SUMMARY

The method of H^+ concentration as used by Michaelis is modified so as to reduce the time necessary for a determination from forty minutes to about two or three minutes.

An electrode which eliminates errors due to O_2 (from oxyhemoglobin) and CO_2, is described. An electrode that may be lowered into the stomach is also described.

A potentiometer reading the H^+ concentration directly, instead of millivolts, is described.

A DIRECT READING POTENTIOMETER FOR MEASURING HYDROGEN ION CONCENTRATIONS

J. F. McCLENDON

From the Physiological Laboratory of the University of Minnesota

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One disadvantage of the use of the hydrogen electrode in determining the hydrogen ion concentration of biological fluids is the time required to calculate the results, and the liability to error by one not experienced in the use of mathematics. In order to avoid part of the calculation, some workers stop the calculation as soon as the logarithm is obtained. These logarithms cannot be used in compiling statistical data in the same way as decimal fractions, and hence the calculation that is saved at one time may have to be done at another. The direct reading potentiometer does the entire calculation for you in zero time.

The difficulty in making a direct reading potentiometer for hydrogen ion concentrations lies in the numerous temperature coefficients that would have to be considered in making an extremely accurate and universal apparatus. But I have shown in a recent paper[1] the temperature coefficient of the whole apparatus is negligible in modern heated rooms with wall thermoregulators. During the summer the temperature variation would be serious only for determinations on blood, where the differences observed are comparatively minute. The error due to temperature is certainly not greater than the same error in reading a burette. The instrument is correct at 23°.

The potentiometer is made to be used in connection with a calomel electrode filled with crystals of KCl, because this avoids the danger of error due to the change in concentration of the KCl solution by diffusion or evaporation. A Weston cell is used. If it does not give the theoretical voltage, it should be standardized and its voltage marked on it.

The potentiometer may be made in two sizes, a large size that is easily made accurate and a small size with evident advantages.

[1] McClendon: This Journal, 1915, xxxviii, 180.

The large form is essentially a resistance wire 23,000 mm. long, with movable contacts. Suppose the Weston cell is correct, that is to say, the voltage marked on it is 1.0185 at 23° the temperature of standardization of the potentiometer, if the current from it is passed through 10,185 mm. of the wire each centimeter will have a fall of potential of 1 millivolt. A storage cell current is sent through and balanced against the Weston cell current by changing the length of the wire. Since the lead storage cell gives a current from about 1.85 to 2.23 volts, the length of the wire should be capable of being changed a corresponding amount. By means of the half dial, *C*, and the sliding contact, *D*, figure 1, the

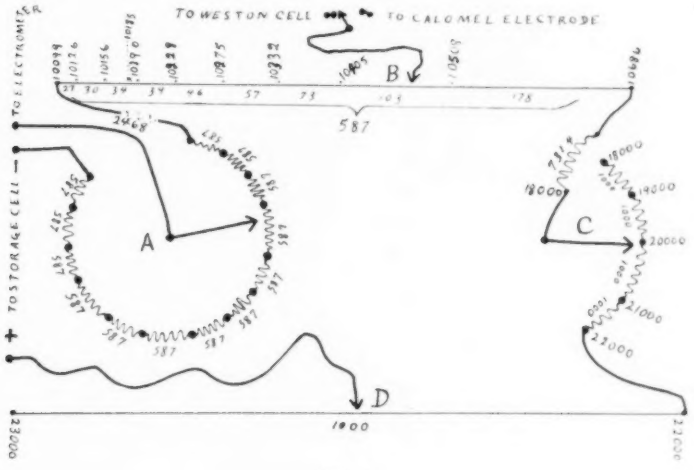

Fig. 1

length of the wire can be changed from 18,000 to 23,000 mm. corresponding to 1.8–2.3 volts. The slide wire, *D*, is 1 meter and hence out of proportion in the figure. In the figure, the length of wire between two buttons is marked by a number parallel to the wire and the total length of wire up to a button is marked perpendicular to the wire. The measurement of the wire for the half dial and slide wire, mentioned, need not be accurate, since the results are not affected by these measurements.

In order to determine the E. M. F. of the hydrogen + calomel electrodes, they are connected with the contacts on the dial, A, and the

slide wire, B. Since with a normal solution of hydrogen ions in the hydrogen electrode, the apparatus has a voltage of 0.2468, therefore 2468 mm. of wire are included between the dial and the slide wire. Since the moving of the decimal point in the hydrogen ion concentration one place, causes a change in voltage of 0.0587, there are 587 mm. of wire in the slide wire, B, and the same length between each pair of buttons on the dial, A. There are 14 buttons, and hence the length of the wire up to the slide wire is $(13\text{x}587)+2468=10{,}099$, and hence 86 mm. on the slide wire is the place for leading off to the Weston cell, or a total of 10,185 mm. A temperature scale may be made for the Weston cell. It is 10,186 at 20°, 10,184, at 25°, and 10, 181 at 30°.

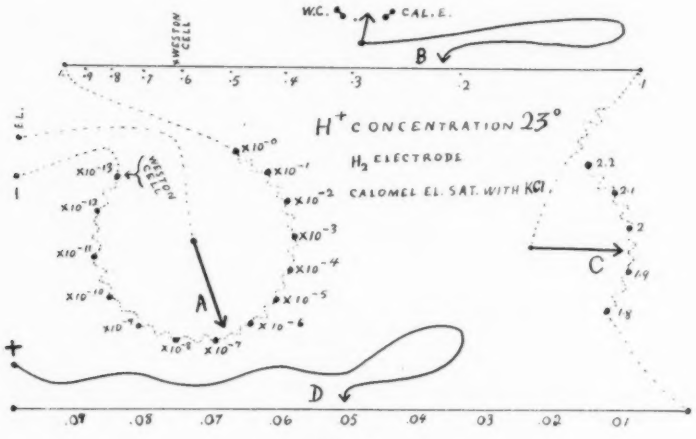

Fig. 2

The scale under the slide wire, B, for reading the hydrogen ion concentration, is logarithmic, and expressed in millimeters is as follows: $27+30+34+39+46+57+73+103+178=587$.

There is a binding post for the negative and one for the positive pole of the storage cell, one to the capillary electrometer, one for the Weston cell and one for the calomel electrode, and a double throw switch to connect the slide, B, either with the calomel electrode or the Weston cell.

A diagram of the face of the potentiometer is shown in figure 2. After the apparatus is set up in the usual manner, the contact of the dial, A, and the slide, B, and the double-throw switch are moved to the

points marked Weston cell, and the half dial, C, and slide, D, moved until a balance is obtained. Then the switch is thrown over and the dial, A, and slide, B, moved until a balance is obtained. The hydrogen ion concentration is then read off directly, for instance 0.4 on the slide wire and $x10^{-2}$ on the dial, or $0.4x10^{-2}=0.004$. Since it saves paper to write $0.3x10^{-12}$, for instance, than the equivalent decimal fraction, the reading is in the most convenient form for general work, without any calculation whatever. The range is from 10^{-0} to 10^{-14}, but this may be increased on the acid side as high as it is possible for the acidity of any solution to rise, in case it is desired to use the method on strong solutions of mineral acids.

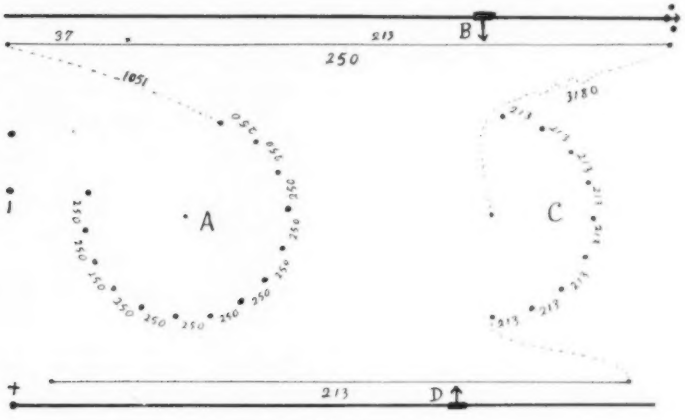

Fig. 3

The lengths of wire in millimeters for the small instrument are shown in figure 3. The length of the slide wire, B, is 250 mm. This is the same length as the logarithmic scale on small-sized slide rules, and logarithmic coördinate paper of this length may be obtained of Keuffel and Esser or other dealers. Therefore it is not worth while to calculate the divisions on the scale. Some samples of logarithmic paper are a little short, especially in a very dry room, but they may be moistened until they expand to the required length and then glued tight, especially at the ends, under the slide wire. By comparing figure 2, the orientation and labeling of the scale may be easily done. The mark on the slide wire scale for the Weston cell is 37 mm. from the left end. The

temperature correction for this point is so small that it may be judged with the eye. In general this mark should be shoved approximately 1 mm. to the right for 5° fall and 1 mm. to the left for 5° rise in temperature. At 30° it should be about 1.5 mm. to the left.

Instead of flexible wires to connect the sliding contact, *B*, with the double throw switch, and the sliding contact, *D*, with the binding posts, it is better to use brass rods with movable sleeves bearing the contact points, as in figure 3.

There are 250 mm. of wire between each two buttons on the dial, *A*, and 1051 mm. between this and the slide wire, *B*. If this wire is measured accurately the instrument will not need calibration for biological purposes, because the non-uniformity of well made wire, taken fresh off of the original spool, will be small, and notwithstanding slight errors in the wire the instrument will show differences accurately.

There are 9 buttons on the half dial, *C*, with 213 mm. of wire between each pair, and 215 mm. on the slide wire, *D*.

The more resistant the wire the better the storage cell will hold up. Manganin (86 Cu, 12 Mn, 2 Ni) invented by Weston, or Constantan (60 Cu, 40 Ni) of from 32 to 36 gauge, are the best materials for the wire, but German silver will do, especially if it is bare and strung in a ventilated box under the dials to avoid heating effects. Some of the wire should be saved to renew the slide wires when necessary. The connecting wires marked by heavy lines in figure 1, should be heavy copper (resistance free). The leads to the potentiometer do not have to be heavy, as only enough current passes through them to operate the electrometer.

I do not have a contact key on the potentiometer, because this key when released, must short circuit the electrometer, and a telegraph key connected directly with the electrometer, is used.

The small instrument is about 12x7 inches.

SUMMARY

The hydrogen ion concentration is read on the scale, *B*, figure 2, and the position of the decimal point read on the dial, *A*.

ACIDITY CURVES IN THE STOMACHS AND DUODENUMS OF ADULTS AND INFANTS, PLOTTED WITH THE AID OF IMPROVED METHODS OF MEASURING HYDROGEN ION CONCENTRATION

J. F. McCLENDON

From the Physiological Laboratory of the University of Minnesota

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The hydrogen ion concentration of the gastric juice and stomach contents after test meals is very well known.[1] According to Michaelis and Davidsohn[2] hyperacidity means a hydrogen ion concentration of from 0.011 to 0.088; average acidity means 0.028–0.0015 and hypoacidity means 0.00041–0.0000001.

Owing to the number of factors involved in the physiology of the stomach, as elucidated by the extensive work of Cannon and of Carlson, it occurred to the writer that the giving of a scant test meal, poor in protein, and the complete evacuation of the stomach all at once, might not reveal all that could be learned of the acidity of the stomach under normal conditions. Consequently an attempt was made to trace the progress of digestion after a normal meal, by lowering a hydrogen electrode into the stomach, and also by removing a few cubic centimeters every half hour by means of a very small tube with a strainer (bucket) on the end that is swallowed. The new departures in the technique are given in two other papers.[3]

It was found possible to keep the tube in the stomach all day except during the ingestion of the meals. After a little practice it was not very difficult to keep the strainer well in the interior of the main part of the stomach. The acidity at the cardia fluctuated more or less owing to the intermittent entrance of saliva. The pylorus became acid more rapidly than the rest of the stomach. But it is not true that the pylorus is always more acid than the fundus. My observations

[1] Fränkel: Zeitschr. f. Exper. Path. u. Therap., 1905, i, 431.
[2] Davidsohn: Zeitschr. f. Exper. Path. u. Therap., 1910, 398.
[3] McClendon: This Journal, 1915, xxxviii, 180 and 186.

indicate that the stomach contents begin to mix immediately after the ingestion of the food, and that the mixing is very thorough in about two to three hours.

The meals that were eaten were governed by the appetite, except in one case when a scant meal was purposely eaten for comparison. The real efficiency of the stomach to take care of these meals may be determined by finding the time required for the acidity of the main bulk of the food to rise to that degree most favorable to peptic digestion. This may be determined with little discomfort and little or no loss of nourishment.

The usual method of pumping out the entire stomach contents after an abnormal meal and titrating the acids and the salts of weak bases, is not calculated to shed very much light on the digestion. Such a procedure implies that acidity is some indestructible property of certain kinds of matter. The same procedure that is used to estimate matter, such as total nitrogen, cannot be used for studying acidity. In the first place new hydrogen ions are formed during the titration and the original number cannot be estimated in this way. The acidity may be determined more accurately by tasting than by titration. In the second place, the same number of hydrogen ions in the stomach may favor or retard digestion according to how they are distributed. Peptic digestion is most rapid in a hydrogen ion concentration of about 0.03 normal[4] and decreases when the acidity increases or decreases. The total stomach contents after being pumped out and mixed may be at the optimum acidity, whereas in the stomach, part may have been too acid and part not acid enough. It is much better to determine the hydrogen ion concentration of a sample taken from the large part of the stomach, away from the wall, because the main bulk of the food is in this location. Whereas digestion may commence immediately in the pylorus and next to the wall, the quantity of food so affected appears to be small, and general digestion is delayed until there has been a thorough mixing of the food with the gastric juice.

THE REACTION OF THE ADULT STOMACH AND DUODENUM

Figures 1 and 2 show a number of curves, representing the rise in acidity of the adult stomach during digestion. On the ordinates are the hydrogen ion concentrations and on the abscissae are the hours

[4] Sorensen: Biochem. Zeitschr., 1909, xxi, 131; Michaelis and Davidsohn: loc. cit.; Michaelis and Mendelssohn: Biochem. Zeitschr., 1914, lxiv,

after finishing the meals. Curves 1–4 are taken from the same individual to show the effect of meals of varying bulk, and hence varying protein content. Curve 1 was taken after a light meal of milk, curves 2 and 3 after average meals and curve 4 after a heavy dinner. Curve 5, from another individual, was taken after a meal that was not only light but also very poor in protein (chiefly carbohydrates). It is clearly seen that the heavier the meal and the more protein it contains, the more slowly the acidity rises. This is due to the fact that the protein neutralizes the acid and the acidity of the stomach cannot rise very high until all of the protein is changed into acid albuminate.

The curves in figure 2 are all taken from different individuals and in general steeper than those in figure 1. This is due to personal characteristics and not to the protein content. Whether this personal difference is due to difference in rate of acid secretion by the stomach, or difference in the quantity of saliva swallowed, is not clear. Curve 12, showing a very slow rise after a medium meal, was taken from a person having a very sensitive throat, and noticeably large quantities of saliva were swallowed. In the average individual, the tube produces no stimulation of the mucous membranes. Curve 11, taken after a light meal, rises steeply, whereas curve 6 taken from another individual, after a light meal, chiefly carbohydrate, does not rise so steeply.

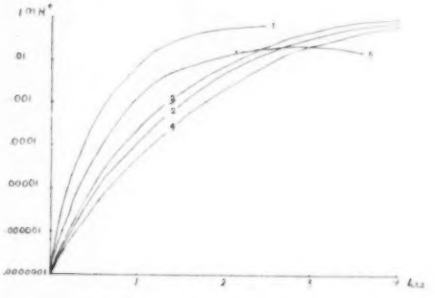

Fig. 1. Curves 1–4, male, 34 years; curve 1, luncheon, 500 cc. milk. Curve 2, breakfast, 1 orange, 250 cc. milk, 200 cc. water, 2 eggs, 2 slices of buttered toast. Curve 3, luncheon, roast lamb, potato, bread and butter, lettuce, ice cream. Curve 4, dinner, soup, beefsteak, potato, bread and butter, tomato, 250 cc. milk, 200 cc. water, slice of pie. Curve 5, male, 32 years, breakfast, bacon, cereal, bread and butter.

It is well known or generally assumed that the height to which the acidity rises is a personal characteristic. This is well illustrated in curves 1–4 (all from the same individual) all of which reach the same maximum. Curves 7 and 8 are from two brothers and show the same maximum. This suggests that the acidity of the stomach is influenced by heredity.

After reaching the characteristic maximum, the acidity of the stomach remains constant for considerable time, at least until the greater part of the food has left the stomach. After this it is difficult to take a sample from the interior, that is, away from the wall, and fluctuations that are sometimes observed may be due to this fact. After all of the food has left the stomach the acidity may fall, due to cessation or decrease in the secretion of acid and also to the swallowing of saliva that is about neutral in reaction and contains protein capable of neutralizing acid.

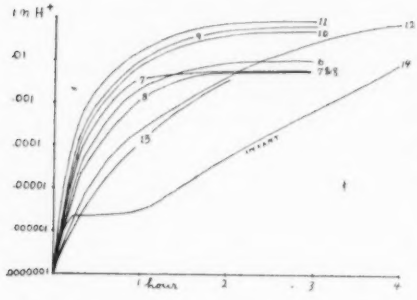

Fig. 2. Curve 6, male, 40 years, breakfast, toast and buttermilk. Curve 7, male, 24 years, dinner, ham, eggs, potato, pie. Curve 8, male, 19 years, dinner, veal roast, rhubarb, soup, buttermilk. Curve 9, male, 18 years, luncheon, 4 roast beef sandwiches, chocolate creams. Curve 10, male, 22 years, dinner, roast beef, 2 eggs, bread and butter, strawberries. Curve 11, male, 25 years, luncheon, ham, potato, bread and butter, coffee, sauce. Curve 12, male, 25 years, ham, potato, bread and butter, sweets, milk, rhubarb. Curve 13 (unfinished), female, 19 years, luncheon, vegetables, bread and butter. Curve 14, compiled from 27 samples of stomach contents of infants of the first month.

The hydrogen ion concentration of a number of duodenal samples taken with the duodenal tube were all very close to 2×10^{-8}. This may be generally true of all normal individuals, but it is not always true in disease. A sample which I determined for Dr. Schneider was very far from this value, but I will not give it here because he may wish to work up the subject from the pathological side.

THE CALIBRATION OF INDICATORS

During the experiments described, I took occasion to calibrate a number of indicator solutions and papers. Michaelis and Davidsohn[5] give a table of indicator solutions that are to be used after the Ewald breakfast. My results, after normal meals correspond closely with theirs except in case of methyl violet (and tropaeolin 00). Since my results correspond closely with those of Sorensen on non-protein solutions, this difference is not due to the higher protein content of my samples,

[5] Davidsohn: Zeitschr. Exp. Path. u. Therap., 1910, viii, 398.

and some of my samples had as low a protein content as those of Michaelis. The difference is probably due to the fact that Michaelis' samples were highly colored with tea. The yellow tea and blue indicator gave a green at 0.01 whereas in my experiments green never appeared, and in those of Sorensen it appeared at 1 normal and yellow appeared at 2 normal. I confirmed Sorensen's results on non-protein solutions and used different brands of methyl violet, Merck's 3B and Grubler's 5B, on gastric contents, but never obtained green at 0.01 when the contents were originally colorless. A similar difference was observed in the case of tropaeolin 00. In my tests it became orange at 0.01 and in those of Michaelis at 0.0033.

This trouble with the color of the sample increases as we go into the duodenum or test the urine. For this reason I calibrated a number of indicator papers to see if such a thing were possible. It is generally considered that indicator papers are less sensitive than the solutions. One reason for this is that the paper used contains substances that preserve the reaction (alkaline earths?). Bausch and Lomb congo paper begins to turn at 0.001 whereas some that I made begins to turn at 0.00005. I made a number of papers by soaking Schleicher and Schull's ash-free paper no. 589 in the indicator solutions and drying, and found them but little less sensitive than the solutions. The end of the paper is dipped into the sample and held there a little while. As the sample rises in the paper any coloring matter is adsorbed very quickly and prevented from rising further. The acid or alkali rises higher and gives the characteristic tinge to the indicator. Pure water rises still higher, so that only the middle of the wet region should be observed. Proteins, and some other interfering substances also, are partially held back from ascending in the paper.

The original solutions and the color of the papers were as follows: methyl violet 0.2 per cent (violet), tropaeolin 00 2 per cent (deep yellow), dimethylamidoazobenzole 0.5 per cent (light yellow), Congo red, 0.5 per cent (red), methyl orange 2 per cent (yellow), alizarine sodium sulphonate 0.5 per cent (salmon pink), p-nitro phenol 2 per cent (light yellow), litmus 2 per cent (violet), neutral red 0.5 per cent (red).

The following table gives the observed changes in adult gastric contents: (−) = no change, B = blue, G = green, O = orange, R = red, V = violet, W = white and Y = yellow, (+) = a slight change:

Normal H^+.....	0.05	0.01	0.005	0.001	0.0001	0.00001	0.000001	0.000005
Met. violet.....	B	B	–	–	–	–	–	–
Tropaolin.....	R	O	–	–	–	–	–	–
2 met.a.a.ben....	R	R	O	–	–	–	–	–
Congo.....	B	B	B	V	V	–	–	–
Met. orange.....	R	R	R	R	O	–	–	–
Alizarine.....	Y	Y	Y	Y	Y	–	–	–
Litmus.....	R	R	R	R	R	+	–	–
P-nitro-ph.....	W	W	W	W	W	+	–	–
Neut. red.....	–	–	–	–	–	–	–	O

These indicator papers were tried on infants' stomach contents with the same result.

The indicator papers used on duodenal contents were: neutral red 0.5 per cent (red), rosolic acid 0.5 per cent (orange), cyanin 0.5 per cent (blue) phenol-phthalein 0.5 per cent (colorless). All of the normal duodenal contents investigated (0.00000005–0.00000001) affected the papers in the same way. Neutral red was changed to orange, rosolic acid was reddened, cyanin and phenol-phthalein were unchanged.

THE REACTION OF THE INFANT STOMACH AND DUODENAL CONTENTS

The work on the infant was done at the request of Prof. J. P. Sedgwick, head of pediatrics, University Hospital. The determinations of acidity were done by me with the hydrogen electrode, except for a few that were done by Dr. Rood Taylor under my direction. The samples were taken by Dr. Taylor, and the radiographs taken by Dr. F. S. Bissell, to both of whom my sincere thanks are due. Dr. Sedgwick gave me some references to the literature.

It was observed by Huenekens[6] that the acidity of the infant's stomach depends on the diet, and Hess,[7] that the titratable acidity of the stomach of the new born before it has taken any food, is high. I was able to obtain several samples of the latter and the acidity was 0.005, but the acidity of the empty infant's stomach is in general high, as will be shown later. Since the protein of the milk binds the acid, we should expect the acidity to be higher the less food is in the stomach, and such was found to be the case. In other words, the regulatory mechanism of gastric acidity in the infant is very imperfect.

Although 27 samples of the gastric contents of infants of the first month were investigated, I had no control over the manner in which

[6] Huenekens: Zeitschr. f. Kinderheilk., 1914, xi, 297.

[7] Hess: Amer. Journ. Diseases of Children, 1913, vi, 264.

the samples were taken, and an actual curve of the rise in acidity cannot be drawn. The entire stomach contents were pumped out and mixed in each sample. Since no relation between age of the infant and acidity was found, the various determinations may be used to construct a theoretical curve. Individual variations cannot be investigated, owing to the few determinations made on one infant. If we consider all samples taken one hour after nursing, for instance, it would be incorrect to use the mean acidity in computing the one-hour point in the curve, because a single stomach that emptied itself in one hour would have more influence than several stomachs that remained full for one hour. But if the variation curve of these determinations is made, the "mode" of the curve may be taken instead of the "mean." Most of the determinations are very near the "mode," and to use the "mode" has the same effect as throwing out the extreme variations. The results are as follows:

Time after nursing in hrs.	0.25	0.5	1.00000	1.5	1.75	2	2.5	3	4
H^+	0.000006	0.000005	0.000006	0.00006	0.00005	0.00012	0.00005	0.001	0.01

The acidity rises to 0.00012, two hours after nursing, and then drops to 0.00005, only to rise again very rapidly until the stomach is empty or until the next meal. This fall is due to the fact that there were not enough samples between two and three hours to obtain a good variation curve from which to determine the "mode." If the acidity curve is smoothed, we obtain the one shown in figure 2, curve 14.

Since woman's milk is neutral[8] and the earliest samples taken were of a much greater hydrogen ion concentration, it is probable that the stomach contains some gastric juice before the milk enters it. The acidity of this juice is high, as shown by curve 14. At the end of four hours there is practically no milk or even curds in the stomach, and at this time the acidity equals that of the adult stomach.

From a quarter to one hour after nursing the acidity remains practically stationary. During this time the protein is being transformed into acid albuminate and all of the acid secreted is used in the process. It was during this period and the hour following it that Davidsohn,[9] and others took their samples and observed the very low acidity. Schackwitz,[10] who did not take samples in the same way as Davidsohn, observed a greater acidity in a few cases.

[8] Davidsohn: Zeitschr. f. Kinderheilk, 1913, ix, 11.

[9] Davidsohn: Zeitschr. f. Kinderheilk, 1911, ii, 420.

[10] Schackwitz: Monatschr. f. Kinderheilk., 1903, xiii, 73.

It seems probable that the milk does not begin to leave the stomach until the end of one hour. From this time on, it is gradually diminished in quantity in the stomach and the acid that it secreted has less protein to neutralize it, and this protein is already partially saturated with acid, so that the acidity of the stomach continually rises. When the acidity has risen high enough for rapid peptic digestion, the quantity of milk left in the stomach is so small and passes out so quickly that it is safe to say that protein digestion practically does not occur in young infants' stomachs after a milk diet.

As a control on the collecting of the samples, the enzymes were determined. Pepsin was determined by the edestin method, trypsin by the casein method and lipase by the splitting of tributyrin as determined by the surface tension measured with Traube's stalagmometer (Michaelis). The stomach contained pepsin and gastric lipase (and milk lipase) but no trypsin.

Twenty-three samples of the duodenal contents of the same babies, taken by means of a catheter, were examined. Some of these were not bile stained but contained trypsin. By means of X-rays and determinations of bile and enzymes and the length of the tube swallowed, the origin of the samples was determined. There is no relation between the time after nursing and the acidity. I exclude three samples that were not bile stained, taken about four hours after nursing in the attempt to get duodenal contents, and showing an acidity of about 0.01. Dr. Taylor considered these as stomach contents, and I have included them in making the curve of stomach acidity.

The variation curve of duodenal acidity has a "mode" of about 0.0008, a maximum of 0.004 and a minimum of 0.00000015. When the stomach is very acid the acidity of the duodenum is lower than that of the stomach, but when the stomach is weakly acid the acidity of the duodenum is higher than that of the stomach. Very probably the acidity of the pylorus is always high, and causes the high acidity of some of the duodenal samples. The less acid samples have a greater admixture of bile. The bile is not sufficient to make the duodenal contents alkaline.

Unless the succus entericus is alkaline and abundant, the entire intestine must be acid. The question arises, what is the nature of the infant's digestion? The enzymes were determined in the same way was those of the stomach. I did not find it possible to distinguish between gastric and pancreatic lipase, but presumably gastric, pancreatic and milk lipase were present. Pepsin was present. Trypsin

was probably always present, although in three samples out of fifteen it seemed doubtful.

It seems certain that a high acidity is necessary for very rapid peptic digestion, but it is probable that after the pepsinogen is once activated by acid, slow digestion may take place at a very low acidity. Sorensen[11] observed peptic digestion in an acidity of abut 0.00008. According to Michaelis and Davidsohn[12] tryptic digestion ceases when the acidity is raised to 0.00001. We see therefore that the acidity of the infant's stomach is high enough to activate the pepsinogen, and that the reaction of the duodenum is as favorable for peptic digestion as it is for tryptic. Probably both forms of proteolysis proceed simultaneously in the infant's intestine. According to Rona and Arnheim[13] the reaction curve for erepsen is about the same as that of trypsin.

Very little is known of these enzymes, except the processes which they hasten and the conditions under which they act. Since it is certain that proteolysis occurs in the alimentary canal of the infant, the next thing to be determined is the reaction of the remainder of the intestine.

SUMMARY

The acidity of the adult stomach rises rapidly during the first 1.5 to 3 hours after a meal, after which it remains stationary until the food has nearly all left the stomach.

The rapidity of rise is less the heavier the meal and the more protein it contains, but it seems also to depend on the efficiency of the individual stomach.

The height to which the acidity rises is a personal characteristic.

The adult duodenal contents are slightly alkaline. The hydrogen ion concentration is about 0.00000002.

The acidity of the infant's stomach (of the first month) rises slowly during the time from fifteen minutes to one hour after nursing, after which it rises rapidly until the stomach is empty. Four hours after nursing it may be as acid as the adult stomach. The acidity is sufficient to activate the pepsinogen, but there is so little milk left in the stomach after the acidity has risen half way that the peptic digestion in the stomach seems unimportant.

The infant's duodenum is more acid than the average acidity of the stomach. Pepsin is always present and peptic digestion must take place.

[11] Sorensen: Biochem. Zeitschr. 1909, xxi, 294.

[12] Davidsohn: Biochem. Zeitschr., 1911, xxxvi, p. 280.

[13] Rona and Arnheim: Biochem. Zeitschr., 1913, lvii, 84.

THE PERFUSION OF THE MAMMALIAN MEDULLA: THE EFFECT OF CALCIUM AND OF POTASSIUM ON THE RESPIRATORY AND CARDIAC CENTERS

D. R. HOOKER

From the Physiological Laboratory of the Johns Hopkins University

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Using a perfusion apparatus the principle of which has already been described (1) the author has sought to perfuse the medullary centers in the dog. The method is now sufficiently advanced to report upon it and in this paper it is proposed to give the results obtained from a study of the effects of calcium and potassium upon the cardiac and respiratory centers.

An investigation of the effects of these salts upon the respiratory activity in the frog (2) while in part apparently conflicting showed clearly that, as compared with a balanced solution, predominance of calcium over potassium caused excitation and predominance of potassium over calcium caused depression. The same effects are now found in the case of the respiratory activity in the dog. It is found further that the salts in question have a definite action upon the tonicity of the cardiac centers, and it is interesting to note that while the resultant effect of potassium is to cause a slowing of both respiration and heart rate the one effect is apparently due to inhibition of one medullary center (respiratory), while the other is due to the excitation of another center (cardio-inhibitory).

The most recent work on the perfusion of the mammalian medulla is that of Herlitzka (3) and of Winterstein (4). Herlitzka fed defibrinated blood to the heart in dogs in which the circulation was limited to the head region. Under these conditions he noted the cardiac and respiratory movements and observed that the repeated head circulation of defibrinated blood, even when oxygenated was inadequate to preserve function for any length of time. The substitution of fresh defibrinated blood, however, temporarily improved conditions. Winterstein perfused new-born rabbits with saline solution at room temperature and observed that the addition of carbon dioxide and other acids produced

respiratory discharge in centers previously quiescent and concluded that the hydrogen-ion concentration of the perfusate governed the function of the respiratory center. Both of these authors review the earlier work in this field. Hirschfelder and Brown (4) have reported on certain pharmacological reactions in the central nervous system perfused with defibrinated blood but their results are not yet accessible to the writer.

Method. Such success as has been obtained by the present method is probably due in large part to the use of a new device for aerating the blood perfusate. The apparatus is shown in figure 1. It consists of an inverted bell-jar covered with a brass plate which clamps air-tight over a rubber washer. Openings in this plate permit the introduction of a thermometer, a tube for the gas mixture and a tube for the entering venous blood; a third tube leads outward through a trap for the escape of gas. In the center of the plate a hollow axle (*a*) carries a hub (*b*) which extends above and below the plate; below, it supports a flat rubber disc (*c*) of a diameter slightly less than that of the bell-jar, and above, a pulley (*d*) rotation of which causes the rubber disc to rotate. As the blood falls upon the rotating disc it is thrown against the side of the bell-jar and runs down in a thin film exposed to the contained air and collects in a suitable reservoir below. (The figure shows only the rubber stopper adapted to close the union

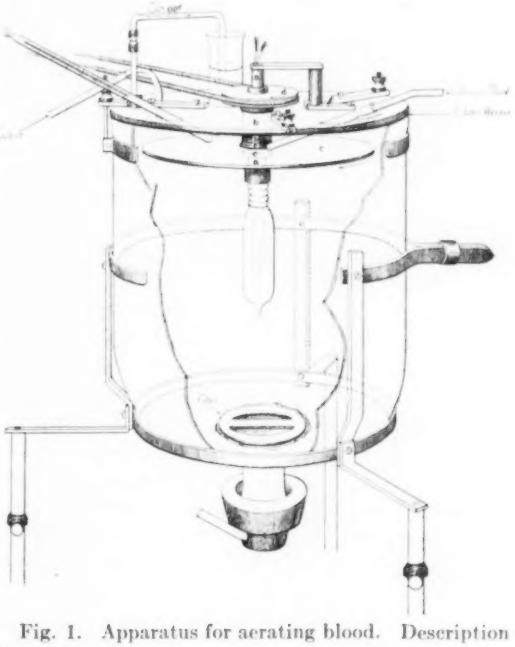

Fig. 1. Apparatus for aerating blood. Description in text.

between the chamber and the reservoir.) The hollow axle is closed by the entrance of insulated wires which lead to a small electric bulb by means of which the temperature is regulated. The bell-jar now in use is about 15 by 20 cm.

In the work here reported the perfusate consisted of washed corpuscles suspended in Ringer's solution. There was in consequence little tendency to froth. In numerous preliminary experiments, however, to test the maintenance of function in the medullary centers and in experiments directed to other ends defibrinated and hirudinized bloods have been used successfully. The apparatus permits the exposure of circulating blood to any chosen gas mixture and obtains adequate aeration without the frothing so likely to occur when protein solutions are agitated. So far as tried the size of the chamber is adequate to experimental needs. It is obvious that a larger one could be substituted if the volume of blood flow demanded it.

Technical procedure. The animal is prepared for perfusion under chloretone anaesthesia. The thorax is freely opened under artificial respiration after ligation of the internal mammary vessels. The right subclavian artery and vein are ligated. Loose ligatures are laid under the superior vena cava and left subclavian artery. The innominate is ligated close to the aortic arch and an inflow cannula is inserted distal to this ligature. An outflow cannula is placed in the right external jugular vein. The preparation is now connected with the perfusion apparatus and the ligatures about the left subclavian artery and superior vena cava are permanently tied. The head circulation is thus isolated except for anastomotic branches along the cord which doubtless unite the spinal branches of the intercostal with the spinal branches of the vertebral arteries. The arterial supply to the brain by the latter paths is insufficient to maintain life. A more serious difficulty lies in the venous anastomoses along the cord by which path there is a continuous seepage of blood from the head circulation into the body. The technical difficulties of occluding this path are so great that it has seemed best to ignore it at least until experimental results are likely to be invalidated by the entrance of chemical substances into the systemic circulation.

After the perfusion is established 300–400 cc. of blood are allowed to waste from the left external jugular vein before the latter is connected to return the perfusate to the apparatus. The isolated circulation as thus accomplished includes the neck parts and the whole head as sup-

plied by the branches of the carotid and the external carotid. The perfusate enters by way of both carotids and the right vertebral artery.

During an experiment artificial respiration is maintained to preserve the cardio-vascular system as a recorder of the medullary effects on this system. The heart-rate has been recorded with a Hürthle manometer connected with the femoral artery and the respiration by recording the movements of the epigastrium.

With the procedure as stated there is a possibility that spinal and vertebral vascular anastamoses might connect the systemic with the isolated circulation and that the former might in part contribute to sustain medullary life. Opposing this possibility are these facts: (a) medullary life ceases at once after ligation of both carotids and both vertebrals or upon stopping the perfusion in an experiment. It is interesting to note, however, that when the medulla has been isolated and perfused for some time the respiratory center will continue to discharge for a little while after the perfusion has been stopped just as the isolated mammalian heart will continue to beat under like conditions. (b) Medullary activity (respiratory movements) continues on the isolated circulation long after the heart has stopped beating. In some experiments lasting an hour or more the heart was not beating at any time.

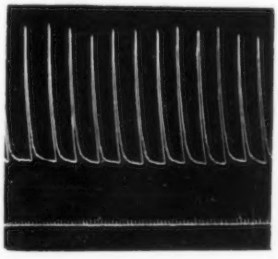

Fig. 2. Experiment of February 10. Tracing of the respiratory movements from a dog in which the head was perfused with defibrinated blood. Record 2 hours, 15 minutes after perfusion was begun. Heart ceased beating 30 minutes and eyelid reflex disappeared 2 hours after perfusion was begun. Perfusion pressure 60 mm. Hg. Upstroke indicates inspiration. Time in seconds.

Figure 2 is reproduced to indicate the reliability of the method employed. It shows the respiratory movements in a dog two hours and fifteen minutes after perfusion was begun. The perfusate consisted of defibrinated dog's blood diluted with about an equal volume of Ringer's solution. The perfusion pressure at the time the record was taken was 60 mm. Hg. Respiratory movements continued regular for ten minutes longer or for a total period of two hours and twenty-five minutes. In this experiment the heart stopped beating in the first half hour, a fact which supports the assertion that the isolation of the medulla is complete.

It is further of interest to note that the eyelid reflex disappeared thirty minutes before the respiratory center ceased to discharge; in the majority of experiments, however, the eyelid reflex persisted longest. It should be stated that this is the longest period the respiratory center has been kept alive. Because the heart had stopped beating no experimental procedures were instituted; this undoubtedly contributed to the period of activity of the preparation because it has been repeatedly observed that changing the experimental conditions in preparations with such a narrow margin of safety markedly reduces the period of survival. Nevertheless a number of experiments were continued successfully for two hours or more. The temperature of the blood in the venous outflow cannula was about 32° C.

Experimental. In the present experiments dogs' corpuscles suspended in salt solution made up the perfusate. An anaesthetized animal was bled, transfused with Ringer's solution and bled again. The blood was defibrinated and the corpuscles were separated in a centrifuge. The corpuscles were then washed twice in and finally suspended in the following solutions:

	per cent		*per cent*		*per cent*
NaCl	0.90	NaCl	0.90	NaCl	0.90
$CaCl_2$	0.03	$CaCl_2$	0.06	KCl	0.06
KCl	0.03				

The solutions, tested before the corpuscles were added, were all slightly alkaline. The Ringer's mixture had a value of $P_H = 8.4+$; the potassium solution $P_H = 8.2$; while the calcium solution fell between the others. This variation in H ion concentration, if it prevailed after the corpuscles were added, was in all likelihood too small to affect the results. Furthermore, it will be noted, the potassium solution which depresses respiratory activity tends to be more acid than the calcium solution which acts as a stimulant.

In order to obtain the requisite volume of perfusate the corpuscles were suspended in a volume of salt solution about twice as great as that of the serum. While this dilution was not determined accurately in the several experiments care was observed to have the dilution the same in the suspensions used in any one experiment. The perfusate as thus prepared was kept on ice over night, in some cases over two nights, before being used. It was hoped that the washing would yield a final solution free of calcium or potassium when the absence of one or the other of these salts was desired so that the results might be correlated

with those obtained from the frog referred to in the introduction of this paper. This was not possible, however, as the supernatant fluid was never free of either calcium or potassium as shown by analysis. The experiments therefore bear only upon the effect of a preponderating amount of calcium and of potassium. The perfusate was aerated in all the experiments with pure oxygen.

Figure 3 shows the stimulating effect of a predominance of calcium. Between the marks the perfusate low in potassium and high in calcium content was substituted for the balanced solution. The respiratory center, previously quiescent, is caused to discharge and the heart-rate is markedly increased. This record, in common with others, shows a latent period of considerable length. The construction of the perfusion system is such that the change of perfusion solution is made some distance from the organ under investigation which no doubt accounts in large part for the delayed effect since some time must elapse before the new solution reaches the medulla. After the return to the control perfusate the respiratory movements quiet down and the heart-rate, somewhat more slowly, returns to the former rate.

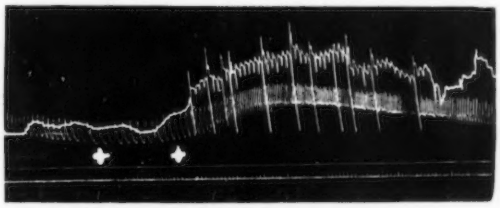

Fig. 3. Experiment of February 9. To show the effect of a preponderance of calcium on the heart-rate and respiratory movements. Downstroke indicates inspiration. Time in seconds.

Figure 4 shows the depressant effect of a predominance of potassium. Between the marks the perfusate low in calcium and high in potassium content was substituted for the balanced solution. A transitory increase in respiratory activity is noted which is followed by a period of complete depression. This momentary increase of respiratory activity was observed in several but not in all the records. The analagous condition of momentary depression when changing to the calcium-strong perfusate was also occasionally seen. This effect is not unlike that sometimes exhibited by the isolated heart when the feeding solutions are changed. Along with the depression of respiratory activity there is a marked slowing of the heart-rate. This slowing is taken to be a vagus effect caused by stimulation of the cardio-inhibitory center since it largely disappears after vagus section. The conclusion therefore

seems inevitable that potassium inhibits one center in the medulla while stimulating another. The data from this series of experiments, in which the cardiac nerves were intact, are presented in Table I.

An analysis of the response of the cardio-inhibitory and cardio-accelerator centers independently of one another was attempted after section of the vagus or accelerator nerves. These results are collected in Table II.

In the single experiment in which the accelerator nerves were cut potassium increase produced a retardation of the heart-rate comparable to that observed in the animals with cardiac nerves intact. The in-

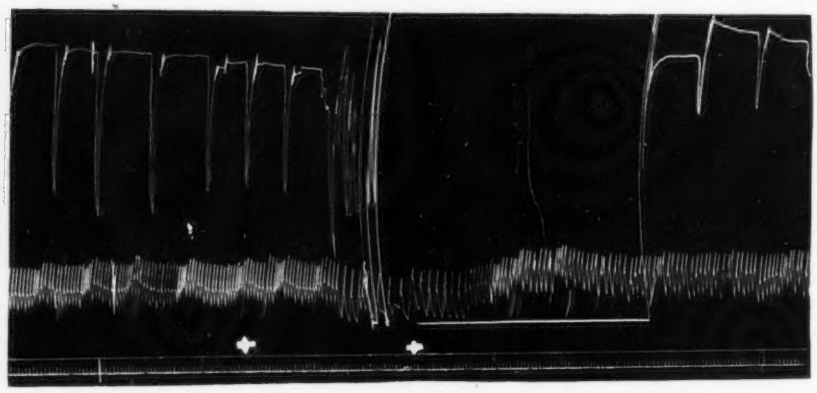

Fig. 4. Experiment of February 2. To show the effect of a preponderance of potassium on the heart-rate and respiratory movements. Downstroke indicates inspiration. Time in seconds.

crease in heart-rate following calcium increase was relatively insignificant. The result is, however, decisive enough to justify the belief that calcium exerts some inhibitory effect on the cardio-inhibitory center.

Five experiments were directed to show that calcium increase stimulates the cardio-accelerator center directly. The difficulty in this procedure is to obtain a slow heart-rate after vagus section so that accelerator effects will be apparent (6). In four of the experiments a slowing of the rate was attempted by reducing the pulmonary ventilation (7); the result in one of these was negative, in two it was insignificant and in the fourth the increase amounted to twenty beats per minute. In the last of these there was a very definite increase in the amplitude of

beat. In the fifth of this series of experiments the heart-rate was slowed by pilocarpine in the systemic circulation. By this procedure (experiment of June 4) a very definite increase in both rate and amplitude of beat was obtained by calcium increase.

TABLE I

Respiratory and heart-rates per minute before and during medullary perfusion of solutions containing a preponderance of calcium or of potassium. Cardiac nerves intact. The records were counted for 30 seconds.

	RESPIRATION		HEART RATE		RESPIRATION		HEART RATE	
	Before Ca	During Ca	Before Ca	During Ca	Before K	During K	Before K	During K
Feb. 2....					3	0	72	30
					18	0	104	30
Feb. 9....	0	52	38	122				
	0	12	34	124				
Mar. 5....					18	0		
					22	0		
					15	0		
Mar. 6....	6	12	70	82	6	0	54	28

TABLE II

Heart-rate per minute before and during medullary perfusion of solutions containing a preponderance of calcium or of potassium after accelerator section and after vagus section. The records were counted for 30 seconds.

	HEART RATE		HEART RATE		PROCEDURE
	Before Ca	During Ca	Before K	During K	
April 7.....	142	150	150	50	After accelerator section
May 1.....	124	134	116	102	After vagus section
May 21.....	102	110			After vagus section
May 22.....	144	164			After vagus section
June 4.....	11	20			After vagus section
	9	24			After vagus section

It seems therefore probable that both cardiac centers are responsive to each of the salts investigated. The effect of calcium is however much more pronounced on the accelerator center while the effect of potassium is much more pronounced on the inhibitory center. The

last described results in conjunction with several negative experiments emphasize further that the marked effect of the salts of calcium and potassium on the heart-rate seen in the experiments in which the cardiac nerves were intact is not due to a direct cardiac effect produced by the entrance of the perfused solution into the systemic circulation otherwise the heart would be influenced as much in the one set of experiments as in the other.

CONCLUSIONS

1. The medulla of the dog may be successfully perfused and the cardiac and respiratory centers will continue to function for a period of two hours or more on a saline solution in which dog's red blood corpuscles are suspended.

2. An apparatus is described which appears adequate for the aeration of blood used in perfusion experiments.

3. The effect of calcium predominating over potassium in a solution containing both of these salts is to stimulate the respiratory center and to increase the heart-rate. Conversely potassium predominating over calcium inhibits the respiratory center and slows the heart-rate.

4. When the accelerator nerves are cut potassium increase causes pronounced cardiac slowing while calcium increase causes slight cardiac acceleration. When the vagi are cut calcium increase causes definite cardiac acceleration and augmentation while potassium increase causes slight cardiac slowing.

5. The results obtained cannot be ascribed to incomplete isolation of the medulla.

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AN INTERPRETATION OF THE MEMBRANE MANOMETER CURVES AS AFFECTED BY VARIATIONS IN BLOOD PRESSURE

J. D. PILCHER

From the Pharmacological Laboratories, Schools of Medicine, Western Reserve University, Cleveland, and the University of Nebraska, Omaha

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Introduction. While there is a large literature on the subject of the tracings formed by the membrane blood pressure manometer, this does not bear directly on the changes in the membrane manometer curve as related to the different mechanisms by which the blood pressure changes were caused. During the past few years a large number of tracings have collected in the laboratories[1] in which the membrane manometer of the Harvard type has been used. As these tracings contain a large number of curves showing various types of circulatory phenomena, such as vasoconstriction and dilation, hemorrhage, etc., they have been studied with the idea of determining if there were not a fairly definite relationship of this kind; to learn, for example, if such phenomena as vasoconstriction, cardiac stimulation or depression, etc., are not represented by characteristic peculiarities in the manometer curves. It seemed probable that such an interpretation would disclose type curves that would give an indication of the cause of a change in blood pressure, which might be of especial value when other, possibly more exact, data were lacking. This we think has been accomplished.

No reference will be made to the form of the individual pulse record for the paper will be limited largely to a presentation of the modifications in the systolic and diastolic pressures caused by various physiological and pharmacological actions on the circulation. The pulse pressure will also be discussed.

Methods. The tracings were made with a Harvard membrane manometer attached by a Y tube to the carotid artery; to the other end of the tube a mercury manometer was attached, damped to give the mean blood pressure only and to prevent any changes in the membrane manometer that might arise from oscillations of the mercury. The dog

[1] With but few exceptions in the Pharmacology Laboratory at Cleveland.

was the usual experimental animal but occasionally the cat was employed. As the work extended over a period of several years, a number of rubber membranes of different thicknesses were used. This fact precludes an exact study of the relation between the amount or extent of the changes in the systolic and diastolic pressures and the degree of the rise or fall in blood pressure. Another fact that must be borne in mind is that the higher the blood pressure, the greater the tension on the membrane, so that a given impulse would probably cause a lesser change at a high level of pressure than at a low level. Neither of these factors, however, would alter the *type* of the curve, which is the question under discussion.

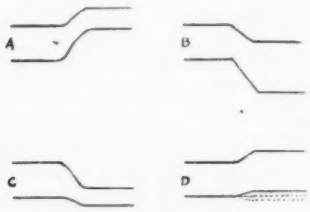


Fig. 1. Type curves formed by the membrane manometer. Upper level, the systolic pressure; lower level, the diastolic pressure. Type A—formed by general vasoconstriction and by increased heart rate. Type B—formed by general vasodilation and by decreased heart rate. Type C—formed by cardiac depression and by decreased volume of blood. Type D—formed by cardiac stimulation and by increased volume of blood.

Experiments showing fling of the lever are disregarded except when this followed the experimental procedure; note will be made of such results.

Method of presenting the results: The results will be presented according to the form of the curve displayed by the tracing rather than by the different physiological or pharmacological procedures. This classification seems to be somewhat simpler as most of the curves fall into definite group types. In all curves the upper limit represents the systolic and the lower limit the diastolic pressures.

The following type curves include the great majority of the curves:

Type A—In this type both systolic and diastolic pressures are elevated, but the diastolic much more than the systolic pressure, so that the amplitude of the pulse excursion is lessened (fig. 1a).

Type B—Both systolic and diastolic pressures are lowered, but the diastolic more than the systolic pressure, so that the amplitude of excursion is increased (fig. 1b).

Type C—Both pressures are lowered, but the systolic more than the diastolic pressure, so that the amplitude is lessened (fig. 1c).

Type D—The systolic pressure is raised somewhat while the diastolic remains the same or is slightly lowered or raised; in any case the amplitude is usually somewhat increased (fig. 1d).

To discuss the individual curves: Type A curve is formed from two distinct physiological phenomena, viz., vasoconstriction and increased heart rate.

Vasoconstriction. This was produced by several procedures, by faradic stimulation of sensory nerves (sciatic), by asphyxia and by large doses of nicotine and aconite, all of which stimulate the vasomoter center; also by the administration of epinephrin, pituitary extract, ergotσxin and ergot whose primary action is a direct stimulation of the arterioles although cardiac stimulation plays a part in the action. A great majority of the curves from these procedure fall under type A, that is as the blood pressure rises both systolic and diastolic pressures are raised but the elevation of the diastolic pressure is disproportionately great, so that the amplitude of the pulse excursion (pulse pressure) is lessened.

Fig. 2. To illustrate the early stage in the vasoconstriction type of curve. Experiment 33, dog; the upper curve is from the membrane manometer; the middle curve the mean blood-pressure from the damped mercury manometer; the signal line is the zero blood-pressure. 4—Sciatic stimulation; note the moderate rise in pressure, with the diastolic level raised considerably but the systolic very slightly.

The purest example of this class of vasoconstriction is that of faradic stimulation of the sciatic nerve (fig. 3 A), for the cardiac action of the drugs employed complicates their action somewhat, although as their predominant action is on the vessels the results are quite similar to that of pure vasoconstriction. The diastolic pressure is the determining feature of this type of curve, with the systolic pressure playing a secondary part. This point is well illustrated in experiment 33, figure 2. Four separate stimulations of the sciatic nerve caused a moderate rise in blood pressure each time; in each case the diastolic pressure was raised considerably while the systolic pressure remained the same or was very slightly elevated. The experiments were made with normal blood pressure and when the

pressure had been lowered by phenol or curare. A further illustration of the greater importance of the diastolic over the systolic pressure in this type of curve (vasoconstriction) is that as the blood pressure rises on stimulation of the sciatic nerve, the earliest effect to be observed in the curve is the prompt elevation in the diastolic level, usually before any change is noticeable in the systolic level.

Huerthle[1] found that, during the rise in blood pressure from sciatic stimulation in the rabbit, the pulse pressure was diminished. He, however, makes no reference to the importance of the diastolic pressure under such conditions.

Experimental data under type A curve. Faradization of the sciatic nerves: Forty-five uncomplicated experiments were made on 17 dogs; 35 curves were of type A and 9 of type D (increase in amplitude by a disproportionate elevation of the systolic level). The explanation of these exceptional cases is not clear; however the respiratory improvement and, secondarily, the cardiac improvement by the relief of a partial asphyxia may account for the increased systolic excursion. This does not include a large number of curves (19) made under abnormal conditions but all of which followed type A; they will be mentioned later. The experiments were made at all levels of blood pressure from 50 to 160 mm. and in all experiments the pressure rose considerably (from 10 to 50 mm.). While in general it may be stated that the greater the rise in blood pressure, the greater was the elevation of the diastolic level, yet the change in amplitude (pulse pressure) seemed to be fairly independent of the rise in pressure, not only in different but in the same experiments. Probably carefully conducted experiments with this end in view would show a closer relationship between the rise in pressure and the variation in the pulse pressure, but it must be borne in mind, as was mentioned in the introduction, that the higher the pressure the greater the tension on the manometer membrane, and this would tend to lessen the systolic excursion while not affecting the diastolic relaxation; this is a factor that would be difficult to estimate. It seems, then, that the form of the curve is the determining feature of this type of curve and not the extent of the change in the amplitude of the excursion.

Sciatic stimulation during low blood pressure from other procedures. The curve did not differ from that of the normal pressure. This type of curve was met twelve times while the pressure was low from curare, four times from phenol and three times from nitrite. With both curare and phenol there were experiments in which sciatic stimulation caused a rise in mean blood pressure with elevation of the diastolic pressure but without change in the systolic pressure as measured by the membrane manometer.

Asphyxia. Slight asphyxia was induced three times in one animal; each time as the blood pressure rose somewhat the manometer curve assumed the form of type A curve.

Aconite and Nicotine. With the great stimulation of the vasometer center from these drugs, type A curve was met once from aconite and twice from nicotine. In two other experiments with nicotine the pulse excursions were in-

[1] Quoted from Erlanger and Hooker (1).

creased somewhat by the greater elevation of the systolic level. As nicotine stimulates the heart as well as the vasomotor apparatus, this may explain the increase in systolic excursion. In a single instance nicotine paralyzed the vasomotor center; the blood pressure curve assumed type B, typical of vasomotor depression which is to be described later.

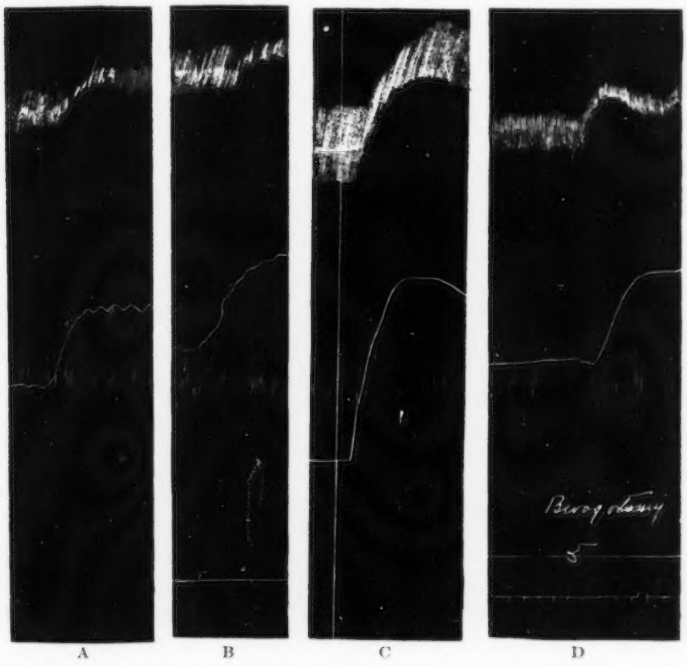


Fig. 3. Type A Curve—To illustrate vasoconstriction and increased heart rate. Upper curve is from the membrane manometer; middle curve is the mean blood-pressure from the damped Hg manometer. A—Sciatic stimulation; experiment 28. B—Injection of pituitary extract immediately after curve A. C—Injection of epinephrin; experiment 34. D—Section of the vagi; experiment 79. A, B and C illustrate the rise in pressure from vasoconstriction; D from section of the vagi.

Epinephrin. The curve of epinephrin resembles that of sciatic stimulation very closely in the majority of experiments (7 of 10). (Fig. 3 C) As the blood pressure rises the diastolic pressure usually is elevated before there is any change in the systolic pressure, and the maximum decrease in the excursion occurs, as a rule, during the rise in pressure; at the maximum level the amplitude of excursion becomes larger but still remains below the normal. This increase in the

excursion at the height of the curve may be due to the cardiac stimulation, for three times the amplitude was above the normal at this point but early in the rise in pressure there was a brief period in which the amplitude was lessened solely by the elevation of the diastolic pressure; these experiments are the three exceptional ones mentioned above so that in reality all the epinephrin curves follow type A. In these exceptional experiments, however, sciatic stimulation also caused an increased pulse pressure. When epinephrin is given in acute cardiac failure, of course the rise in pressure is accompanied by a greater increase in systolic than in diastolic pressure.

Pituitary extract. Type A curve was formed in five of seven experiments; in the other two both pressures were equally elevated; possibly the cardiac element was more marked in these cases. The curve at times resembles that of sciatic stimulation very closely as is shown in figure 3B, in which sciatic stimulation immediately preceded the injection of the pituitary extract. There may be considerable decrease in the excursion solely by the elevation of the diastolic level, with the systolic level remaining the same: in experiment 227, on the injection of pituitary extract the blood pressure rose from 75 to 130 mm. and the excursion was lessened from 24 to 10 mm. solely by the elevation of the diastolic pressure; however, too much stress should not be placed on but one experiment for there may have been a certain amount of fling of the lever that was not noted during the experiment and this would have given a greater original systolic excursion than really existed; the point that is to be emphasized is that the diastolic pressure is the determining feature of the pituitary curve.

Ergotoxin and ergot. Each drug formed type A curve in but a single experiment.

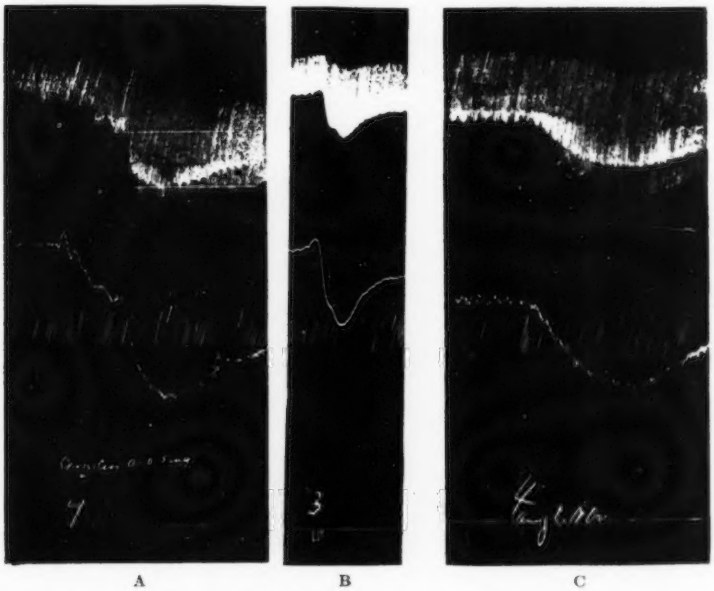
Type A curve from increased heart rate. The general form of the type is maintained in this group but as a rule the blood pressure rose more gradually than in the members of the previous group (especially sciatic stimulation and epinephrin) so that the changes were not so pronounced, although in a few instances there was little difference between the two classes (fig. 3 D). The heart rate was increased by section of the vagi or by the administration of atropin to paralyze the vagal endings.

Vagus section. In nine instances this resulted in an increased heart rate and a rise in blood pressure (average 35 mm.). All manometer curves assumed type A with moderate decrease in the amplitude of excursion, largely by the elevation of the diastolic pressure; occasionally the systolic level was but very slightly changed, if at all.

Atropin. When atropin caused an increased heart rate and a rise in blood pressure, type A form of curve was found in three of four experiments; in the fourth case type D was formed.

Erlanger and Hooker (1) have pointed out that acceleration of the heart, such as occurs after section of the vagi, as a rule diminished the pulse pressure, a statement that these results confirm.

Type B curve. In this type the amplitude of the excursions is increased by a great lowering of the diastolic pressure; the systolic pressure is also usually lowered but exceptionally may remain unaffected. The type is met during the fall in blood pressure from two distinct phenomena, vasodilation and cardiac slowing, conditions that are just the reverse of those that produced the preceding type of curve.



A B C

Fig. 4. Type B Curve—To illustrate the effect of the fall in blood-pressure from cardiac slowing and vasodilation. Upper curve is that of membrane manometer; the middle curve is the mean blood-pressure from a damped Hg manometer; the signal line is the zero blood-pressure. A—Cardiac slowing from cevadin; experiment 49. B—Vasodilation from nitroglycerin; experiment 90. C—Vasodilation from amyl nitrite; experiment 55.

The vasodilator group. The vasodilation was induced most typically and in the purest form by the administration of nitrites—amyl and sodium nitrites and nitroglycerin (fig. 4 B, C); also by chloroform, phenol, atropin (in large doses only), and by the intravenous administration of curare which probably causes peripheral vasodilation. The typical dilator action of chloroform and phenol, however, is modified

by their cardiac action. In this group the heart rate was practically unaffected.

The Nitrites. In all the experiments (19) with the nitrites as the blood pressure fell the diastolic level was greatly lowered, while the systolic level, though usually also lowered considerably, was frequently unaffected or but very slightly lowered; this caused a great increase in the amplitude of the excursion of the pulse-pressure. Too much emphasis should not be placed on the occasionally unchanged systolic level as the sudden emptying of the heart into the dilated vessels would undoubtedly tend to cause fling of the manometer lever. It is rather striking that there seemed to be a tendency for the systolic level to recover to the normal somewhat earlier than the diastolic level, but as many of the experiments were interrupted by other procedures, the evidence for this point is not complete.

There was no material difference in the curves of the three members of the group, amyl and sodium nitrites and nitroglycerin. There were twelve experiments with nitroglycerin (0.5 to 1.0 mgm. per kilogram), five with amyl nitrite and two with sodium nitrite (5 and 10 mgm). In each case there was a pronounced fall in blood pressure, varying from 25 to 65 mm; the experiments were made at all levels of pressure from 70 to 205 mm, without showing any striking differences in the manometer curves. The amplitude was more than doubled in several curves.

Chloroform. This has a twofold action, depressing both the heart and vasomotor systems. When administered not too rapidly by inhalation the vasomotor action predominates; when given by vein in suitable dosage direct cardiac depression can be obtained. The intravenous results will be discussed under the cardiac depressants.

Chloroform was given by inhalation seven times to six animals and in all of them type B curve was formed as the blood pressure fell. In a single experiment, in which the administration was pushed rather rapidly, the systolic pressure fell about as much as the diastolic, thus approaching the cardiac depression type of curve; the blood pressure also fell more rapidly in this experiment. The average fall in pressure was 50 mm., extremes of 20 and 90 mm.

Phenol. As the blood pressure fell from the intravenous administration of phenol in six of seven experiments type B curve was formed, thus indicating that the cause of the fall in pressure was vascular, at least in part, and for this there is direct evidence from the perfusion method elsewhere described (2). Direct cardiac depression is the other factor in the fall in pressure; there is, however, but a single instance in which there is evidence for this statement: in one experiment the systolic level fell more than the diastolic, thus decreasing the amplitude of the excursion in a manner that indicates cardiac depression (to be discussed later). In all experiments the blood pressure fell sharply, an average of 45 mm., extremes of 25 and 65 mm.

Atropin. When atropin caused a fall in blood pressure the curve assumed type B, indicating that the fall in pressure was due to vasomotor depression as the heart rate was not materially affected under the conditions of the experiment.

There were six experiments in which atropin in doses of from 0.1 to 1.0 mgm. per kilogram, and single experiments with 5 and 10 mgm., caused a mean fall in

blood pressure of 15 mm. (extremes 5 and 72 mm.). All but one tracing followed type B. The amplitude was increased a variable amount, usually moderately, but in one experiment from 5 to 35 mm., part of which was probably due to fling of the lever. The heart rate was somewhat increased twice and decreased once; in the other experiments there was practically no change in rate as the vagi were divided or previous doses of atropin had been given.

Curare. During the fall of blood-pressure from the intravenous administration of curare there is always a great lowering of diastolic pressure, and usually, but not always, the systolic pressure falls also; in either case there is a marked increase in the amplitude of the cardiac excursions. The lowering of the diastolic level precedes the fall in systolic pressure, and the former falls abruptly while the latter falls gradually, indicating that the lowering of the diastolic level is the essential cause of the fall in blood pressure. As this curve is of the same type as the nitrite curve it gives further evidence that peripheral vasomotor depression is the cause of the curare fall; peripheral because the vasomotor center is not depressed but rather stimulated (2).

Curare was given a total of 23 times to 23 dogs. The average fall in pressure was 40 mm. (extremes 12 and 95 mm.). In several experiments the systolic pressure was either raised slightly (five times) or remained unchanged (twice); with but one exception the blood pressure fall was considerably less than in the other experiments, so that when the curare action is marked the systolic level also falls. Fling of the lever may also account for the maintenance of the systolic pressure in such experiments. When the minimum blood pressure was reached, usually the systolic pressure had fallen considerably so that the pulse pressure was lessened, although there were numerous exceptions as noted.

Hales,[2] and later Bernard,[2] pointed out that a fall in blood pressure increases the amplitude of the pulse. Our observations confirm this statement as regards the fall in pressure from general vasodilation and from decreased heart rate; we wish to emphasize that the increase in the pulse pressure is brought about largely by the disproportionate lowering of the diastolic pressure. The lesser change in the systolic level is nicely put by Marey:[2] "If we assume that the more or less rapid and more or less abundant entrance of blood into the arterial system result from the excess of ventricular pressure over the pressure in the arterial system, then it is evident that a fall of arterial pressure would be exactly similar to an increase in the force of the heart beat, and there is, therefore, nothing surprising in this augmentation of the force of the pulse in all conditions that lower the blood pressure."

The effect on the pulse curve of lowered blood pressure from hemorrhage will be discussed later.

Type B from decreased heart rate. The curve is similar to that of the vasodilator group except that usually the systolic level is not lessened to as great an extent. The curve is simply that of the "vagus pulse" characterized by a prolonged diastole, during which the pressure falls greatly, while the systolic pressure is but moderately lowered or may

[2] Quoted from Erlanger and Hooker (1).

even remain unaffected; in any case the pulse pressure is greatly increased.

The heart rate was decreased by faradic stimulation of the peripheral end of the divided cervical vagus, by the intravenous administration of veratrum viride and its alkaloid cevadin, and in a single experiment by aconite.

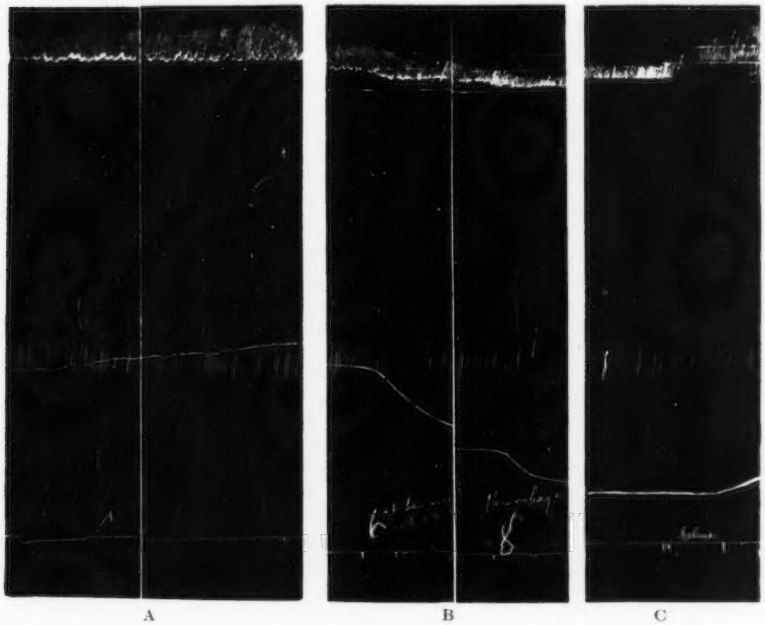
Veratrum viride, as the tincture, was given in doses of from 1 to 10 mgm. per kilo of body weight; cevadin in 0.05 mgm. per kilo doses. In all there were 13 experiments on 11 animals and the two drugs gave similar results. With the administration of the drug the heart rate was markedly decreased so that the blood pressure fell from 30 to 70 mm. in each experiment. All experiments gave type B form of curve (fig. 4A).

Vagus stimulation gave results similar to veratrum in three cases. In other experiments the heart was completely inhibited so that the systolic pressure fell to zero.

Type curves C and D. In the preceding curves the characteristic feature has been the changes in the disatolic pressure; in the curves to be described next (C and D) the characteristic feature is the systolic variation in pressure although the diastolic pressure may also be considerably changed. In type C both levels are lowered, but the systolic much more than the diastolic level so that the pulse pressure is lessened. This type is met typically following sudden arterial hemorrhage and depends upon a decreased quantity of blood (fig. 5 B); and from cardiac depression. In type D the systolic pressure is somewhat elevated while the diastole remains unaffected or is slightly raised or lowered. The curve follows an increase in the volume of blood, either from the injection of defibrinated blood or normal saline solution into the bled animal, or from the injection of saline solution into the normal animal (fig. 5 A, C). Type D is also met from cardiac stimulation, although the experiments showing pure cardiac stimulation are somewhat limited in number. As the two curves follow one another experimentally it is simpler to discuss them together.

Hemorrhage. The animals were bled rapidly from the femoral artery in successive small hemorrhages until a very low point in blood pressure was reached; then either the defibrinated blood or normal saline solution, or each in succession, was injected into the femoral vein. At the onset of the hemorrhage the systolic level usually fell promptly, and the diastolic level also fell but to a much lesser extent, so that the amplitude of the pulse excursions was lessened. This effect was met with small hemorrhages of but 5 cc. per kilo and increased with succeeding hemor-

rhages, the pulse pressure becoming smaller and smaller as the total amount of blood withdrawn increased. The individual experiments showed considerable differences in the extent of the change in the maximum and minimum pressures because of the variation in the thickness of the membrane, so that it is not feasible to make quantitative com-



A B C

Fig. 5. Types C and D Curves—To illustrate the effect of saline infusion and hemorrhage; experiment 18, dog; the upper curve is from the membrane manometer; the middle curve the mean pressure from the damped Hg manometer. Curve A—Saline infusion before hemorrhage. Curve B—Hemorrhage, two portions of 10 cc. each. Curve C—Saline infusion after hemorrhage. Curves A and B have been interrupted.

parisons. Between the successive hemorrhages there was usually very little recovery in the systolic pressure but as a rule the diastolic pressure recovered somewhat.

Reinjection of blood and saline solutions. When the blood pressure had fallen to the minimum point compatible with safety, the defibrinated blood or saline solution were injected. As the pressure rose the ma-

nometer curves showed changes practically the reverse of those formed from the withdrawal of the blood, i.e., while both the systolic and diastolic pressures were raised, the systolic was raised more than the diastolic level so that the pulse excursions were increased. If the injections were sufficiently large, the curve returned to the normal.

The injection of saline solution into the normal animal. This resulted in a constant, though variable, elevation of the systolic pressure; the diastolic pressure was variable, slightly raised, unchanged or lowered a little. The amplitude of the excursions were increased by the predominance of the systolic rise. The slight lowering of the diastolic pressure is of little import as it may have been caused by fling of the lever.

Data on hemorrhage and saline infusion. There were 16 experiments on 12 dogs. The blood was withdrawn in 5 to 10 cc. portions until the minimum point in pressure was reached, about 30 to 40 mm. In 12 experiments the systolic pressure was lowered disproportionately so that type C curve was formed; in one experiment the maximum and minimum pressures were equally lowered and in three the curve was irregular but the diastolic pressure recovered before the systolic which is the usual occurrence. When the defibrinated blood (5 experiments) and saline solution (2 experiments) were injected there was a prompt elevation of the systolic level while the diastolic level was but slightly affected as mentioned above until rather large amounts were injected when conditions returned toward the normal.

Saline infusion into the normal animal. Ten experiments were made on six dogs, injecting from 5 to 35 cc. per kilo. All experiments gave an increased amplitude of excursion after type D curve. The diastolic pressure was slightly raised four times and slightly lowered or unaffected each three times. In each experiment the blood pressure rose moderately, average 13 mm., extremes of 4 and 20 mm.

Type C curve from cardiac depression. Direct cardiac depression was produced by the intravenous injection of chloroform or by saline solution saturated with chloroform. The drug, of course, depresses the vasomotor system as well, but by giving small doses by vein the primary action seems to be direct cardiac depression as the action is of such brief duration and recovery prompt and complete; in one instance, however, there was evidence of marked temporary depression of the vasomotor center without altering the cardiac depression form of curve. On the injection of 0.1 to 0.2 cc. of chloroform the blood pressure fell immediately and considerably, but returned to the normal usually within two to four minutes. During this period the cardiac excursions were greatly lessened, largely by a great lowering of the systolic level although the diastolic level was also lowered somewhat (fig. 6). Usually, also,

the diastolic pressure recovered before the systolic, which indicates weakening of the heart. The heart rate was not materially affected except in three instances when there was considerable slowing without altering the type of the curve. There were ten such experiments on six animals. The blood pressure fell an average of 45 mm., extremes of 20 and 67 mm.

Cardiac depression was also observed in ten experiments from *asphyxia* when the vasomotor center was paralyzed, thus preventing the usual rise in pressure from central vasomotor stimulation. In each instance as the pressure fell there was a gradual lowering of the systolic pressure before the diastolic pressure fell as well, thus following type C curve.

Fig 6. To illustrate cardiac depression. Experiment 91, dog; the upper curve is from the membrane manometer; the middle curve the mean pressure from the damped Hg manometer; signal line is the zero pressure. At the signal chloroform in saline solution was given intravenously.

Experiments showing *cardiac stimulation* are suggestive only, for they are not free from the element of vasomotor action. Thus strophanthus gives the curve of vasoconstriction somewhat more frequently than what may be considered the type of cardiac stimulation, that is with the systolic level raised more than the diastolic level, thus increasing the excursion. With caffein the number of experiments with good membrane manometer curves is limited and these show a variable response: In two instances stimulation was indicated by a rise in blood pressure, the amplitude of excursion was either increased by a greater elevation of the systolic level or it remained unchanged with both pressures somewhat raised, both curves indicating cardiac stimulation. In other experiments, with fall in pressure, the systolic level was twice lowered more than the diastolic level, indicating cardiac depression; in still another instance vasodilation was indicated. In the acute fall in pressure from the intravenous injection the curve was also irregular, indicating either cardiac or vasomotor depression or both.*

The irregularity of the caffein curves is readily appreciated when it is recalled that the drug primarily stimulates the heart and depresses the vasomotor apparatus and later depresses the heart as well.

Discussion. After the experimental results had been classified as in the text, the explanation of the formation of the type curves was sought on *a priori* grounds and are here presented. The main factor in the formation of the curves is, of course, the physiological processes, modified, it may be to a certain extent, by the mechanical influences of the rubber membrane of the manometer. As the various types of phenomena were classified in the text as if they occurred in what may be called the pure state, that is, unmodified by other circulatory influences, the same plan will be followed in this discussion. It must be borne in mind, however, that the various factors that determine the circulation interact with, and modify, one another, so that probably a strictly "pure" type does not exist.

The vasoconstriction type. This is characterized by the disproportionate elevation of the diastolic level. The diastolic pressure is determined by the rate of blood-flow through the arterioles to the venous side of the circulation, therefore the greater the resistance offered by the arterioles during the vasoconstriction, the less will be the passage of blood through them and consequently the higher the diastolic pressure will rise. The systolic pressure is determined by the rate and force of the discharge of blood into the aorta; the greater the resistance in the aorta the smaller the quantity of blood that can be forced into it with a given strength of heart beat. The higher the diastolic pressure rises the nearer it approaches the systolic intracardiac pressure, and this means that with each systole a lesser quantity of blood will be discharged into the aorta because of the increasing resistance to the discharge, and the added increment of pressure will be less and less as the diastolic pressure rises. Therefore, as was stated in the text, it is the diastolic pressure that determines the vasoconstriction type of curve, with the systolic pressure playing a secondary part. The mechanical factor that might influence the form of the curve is that the higher the blood pressure the greater the resistance offered by the rubber dam of the manometer. This would tend to lessen the systolic excursion while not affecting the diastolic relaxation. However, as this type of curve is the same at all levels of blood pressure and with different manometer membranes, the mechanical factor is evidently of little importance in determining the type of curve.

The vasodilation type, characterized by excessive depression of the diastolic pressure is lowered by the rapid escape of blood through the dilated arterioles into the venous side of the circulation. The systolic pressure tends to approach the normal as the expulsive force of the heart remains

practically unchanged and because of the lessened resistance in the aorta to the systolic discharge. Thus the determining feature of the vasoconstriction curve is the diastolic pressure.

Cardiac depression. The systolic level is lowered by the lessened force of the cardiac contraction. The diastolic pressure is primarily unaffected as the resistance to the blood-flow from the arterial to the venous side is primarily unaffected.

Cardiac stimulation. The systolic level is raised because of the increased force of the systolic contraction. The diastolic pressure is not materially affected.

Change in the volume of blood. Decrease in the volume of blood would result in a lessened discharge, hence a lowered systolic pressure. Increase in the volume would result in a greater systolic output and a higher systolic pressure. In either case the diastolic pressure would be a secondary feature. This agrees with the experimental findings. The change in the systolic pressure was greater in the case of hemorrhage as the compensatory mechanism would not be as efficacious as when the volume of the blood is increased by the addition of saline solution. When the infusion followed hemorrhage the increase in the systolic level was correspondingly great.

To recapitulate in another way. The diastolic pressure is determined mainly by the outflow through the arteries during the diastolic pause and it is, therefore, affected mainly by the calibre of the vessels and by the rate of the heart; so that changes which affect mainly the diastolic pressure are either vasomotor or heart rate. The systolic pressure is determined mainly by the pressure with which the heart forces blood into the vessels and is, therefore, determined by the force of the cardiac contraction and by the volume of blood at the disposal of the heart; so that changes in the systolic pressure implies either variations in the force of the cardiac contractions or in the volume of the blood at the disposal of the heart.

Conclusions. The membrane manometer gives evidence of the cause of a variation of blood pressure by the form of the curve assumed. These curves are classified in the text.

Vasoconstriction is indicated by the elevation of both diastolic and systolic pressures, but the former much more than the latter, so that the amplitude of the excursion (pulse pressure) is greatly lessened.

Vasodilation is indicated by a lowering of both pressures, with the diastolic pressure lowered much more than the systolic, so that the amplitude is greatly increased. The diastolic pressure is the determining feature in both vasoconstriction and dilation.

When the heart rate is increased sufficiently to raise the blood pressure, the curve resembles vasoconstriction; while a lowered pressure from decreased rate is similar to that of vasodilation.

Following hemorrhage both systolic and diastolic pressures are lowered, but the systolic disproportionately, so that the amplitude of the excursions are lessened. The infusion of blood or saline solution increases the excursion largely by elevating the systolic level. In both hemorrhage and infusion the systolic pressure largely determines the form of the curve.

I am glad to express my indebtedness to Dr. Sollmann for many helpful suggestions, especially in the discussion of the results.

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STUDIES ON THE HYDROGEN-ION CONCENTRATION IN BLOOD UNDER VARIOUS ABNORMAL CONDITIONS

M. L. MENTEN, M.D. AND G. W. CRILE, M.D.

From the Cushing Laboratory for Experimental Medicine, Western Reserve University, Cleveland, Ohio

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INTRODUCTION

Since the application by Höber (1) in 1900 of the Nernst hydrogen concentration chain to the measurement of the reaction of the blood, our knowledge of that subject has been greatly extended. Many modifications of the original method have been adopted by various investigators, but practically all the results published reveal the fact that the reaction of normal blood lies within comparatively narrow limits, varying according to the earlier observers between $0{,}3.10^{-7}$ and $0{,}7.10^{-7}$ (2) and according to the later and more accurate studies of Hasselbalch (3) on Mammalia at body temperature between $3{,}5.10^{-8}$ while Michaelis (4) records an average value of $2{,}75.10^{-8}$ at a temperature of from 18°C to 20°C. Both these later observers have noted that venous blood is always slightly more acid than arterial blood, due, probably, to the greater carbon dioxide tension in the former.

Even the investigations of blood in pathological conditions, where acidosis occurs, have failed to show any considerable deviation from the narrow limits already quoted, and only in diabetes, (5) (6) where deep coma has been reached, does there occur any demonstrable increase in the hydrogen-ion concentration of the blood, although oxybutyric and diacetic acids are known to be produced in not inconsiderable amounts in that disease. The only remaining conditions in which an increased acidity of the blood has been observed, where the technique is free from serious criticisms is that of narcosis, reported by Michaelis (7).

The investigations outlined in the following pages deal with changes in the hydrogen-ion concentration, noted in animals in various conditions.

METHODS

The method employed for estimating the acidity of the blood was that of measuring the hydrogen-ion concentration by means of the gas chain; and since the technique used was essentially that already published in detail by Michaelis (8) no further description need be given here. It may be mentioned however that all measurements were made on animals at laboratory temperature varying from 18°C to 24°C., and for these variations corrections were made. The quantities of blood used varying from 2.5 cc. to 3.5 cc., were diluted to 6.5 cc. with 0.85 per cent sodium chloride containing hirudin in solution. When possible the blood was allowed to drop directly into the electrode from the ear of the animal employed; in other cases a cannula was inserted in the vessel and the blood passed from this into the electrode. In every experiment duplicate readings were made.

Instead of expressing our results as the hydrogen concentration $C_H = 10^{-n}$ as has been done in the introduction, we have followed the Sörensen procedure in using throughout the hydrogen-ion exponent pH = p.

RESULTS AND DISCUSSION

In some of our earlier estimations of the hydrogen-ion concentrations of the blood it was observed that in anaesthetized animals the acidity of the blood was considerably higher than that reported for normal animals. A systematic study was then made of the blood of animals anaesthetized with chloroform, ether and nitrous oxid. For these experiments dogs and rabbits were chosen because of the comparative ease in obtaining blood from the ear blood vessels of these two animals. As previously stated the blood was bled from an incision in the blood vessel of the ear directly into the electrode; the same animal was then anaesthetized and blood again taken from the same site in a similar manner so that in every case the blood in anaesthesia could be compared with the normal blood of the same animal.

In a large number of experiments the values obtained for normal blood show a wide variation, ranging in the dog from pH = 7.64 to pH = 7.32. In the rabbit the variations are even more marked, the minimal and maximal figures obtained being pH = 7.18 and pH = 7.70, a phenomenon which will be discussed later.

Under the influence of the three above mentioned anaesthetics, when anaesthesia reaches the stage in which the reflexes are completely

abolished, the reaction of the blood measured at 20°C. may fall to pH = 7.00. Although our experimental studies of the blood reaction in anaesthesia number over twenty-five, no protocols are recorded here because the degree of acidity produced is, as far as could be ascertained, dependent solely upon the amount of the drug inspired, if corrections are made for the different weights of the various animals. Corresponding more or less closely to the degree of anaesthesia therefore, values extending from pH = 7.00 to pH = 7.60 may be obtained. The deeper the anaesthesia the more marked the acidity, and vice versa; also prolonged duration of the anaesthesia is apparently not a factor in increasing the hydrogen-ion content of the blood. Further, the increase in acidity commences as soon as the anaesthetic enters the circulation, and a diminution takes place immediately on the cessation of the administration of the drug, until 45 minutes subsequently the blood again resumes its normal hydrogen-ion concentration.

Narcosis produced by morphia, even when this drug is given in such large doses as 75 cc. of 1 per cent morphia sulphate in two hours, fails to reveal any demonstrable changes in hydrogen-ion concentration.

As it is interesting on account of the prolongation of the duration of the induced acidity, mention is made of one experiment with ethyl alcohol, where a dog whose normal blood reaction was pH = 7.47 was given by stomach tube a mixture of 60 cc. of 95 per cent alcohol in 40 cc. of water. Forty-five minutes subsequently the blood acidity was pH = 7.32 and 6 hours later, although the animal had apparently gained complete consciousness, the reaction remained at the high value of pH = 7.28.

Finally, in two cases of surgical operations in the senior author's clinic, during nitrous oxide anaesthesia, measurements showed the blood reaction to be respectively pH = 7.20 and pH = 7.22.

Not only in anaesthesia but also in other abnormal states the blood reaction was observed to become more acid. The most striking of these was fright, a phenomenon remarkably shown in the rabbit. Ordinarily if blood is taken from the ear of a rabbit, so that the animal is disturbed as little as possible, the blood shows a fairly high alkalinity. Under the influence of fright this is altered in an astonishingly short time and to a most profound extent. Another noteworthy feature is the rapidity with which the blood regains its normal reaction. The following protocols will serve to illustrate these facts:

I. Normal blood reaction of rabbit was pH = 7.67. Animal was comfortably placed in a box covered with a wire screen and frightened

for ten minutes by a barking dog. Blood immediately taken from the ear blood vessels showed an acidity of pH = 7.17. The rabbit was then allowed to rest quietly 45 minutes and again frightened, directly following which the animal was bled, and the blood reaction was found to be pH = 7.34. After being allowed to rest quietly for 30 minutes, blood examined in like manner gave a value of pH = 7.60.

II. Rabbit whose normal blood was pH = 7.60, after being frightened for 5 minutes in the manner above described, showed a hydrogen-ion concentration of pH = 6.98. Fifteen minutes subsequently the blood again gave a normal reaction. No signs of distress other than shallower and more frequent respirations seemed to accompany the increased acidity and the animal apparently suffered little inconvenience during or subsequent to the fright and 15 minutes afterward appeared to be quite normal again. It is probable however that such a process frequently repeated throughout life must leave some traces. It is indeed, most probable that the varying figures obtained for the normal blood of the rabbit noted earlier in this paper, and which have been previously reported by Hasselbalch and Lundsgaard (9) have their explanation in the above phenomenon, the repetition of which during the life of the rabbit may account for the varying pathological conditions of the blood vascular apparatus of that animal, encountered by so many investigators and which, therefore, make it such an undesirable experimental animal. Similar observations regarding such marked effects of fright have been very rarely observed by us in dogs.

Since the stimulus of fright can produce such a prompt and intense response it is to be surmised that in other emotional disturbances, similar changes in acidity would be encountered. Experiments to test this supposition were made for anger in cats, and it was found that the expression of that emotion is also accompanied by profound changes in the blood of the animal. Unfortunately the cat is not as desirable for such experiments as the rabbit. To obtain blood from the cat without general anaesthesia is much more difficult than from the rabbit, largely because in the former the blood vessels must be exposed under local anaesthesia. Further if the operation is not skillfully and quickly done so as to minimize the pain and eliminate as far as possible the excitement of the animal, the blood from the beginning assumes the high acid value indicative of emotional disturbance. The following abbreviated protocols serve to illustrate the character and results of the experiments.

I. Normal blood withdrawn by syringe from the femoral artery, exposed under cocaine, had a value of pH = 7.43. The cat was angered

by a barking dog and in blood then withdrawn from the femoral artery acidity was pH = 7.20. The animal was kept tied down and at the end of an hour the blood reaction was pH = 7.37.

II. Cat was tied down and femoral artery exposed under cocaine. Normal blood withdrawn with syringe had a hydrogen-ion concentration of pH = 7.20. Cat was then frightened for 15 minutes and blood acidity had risen to pH = 7.02. It may be noted, that in these experiments on cats the normal reaction of the blood acidity is invariably high, due, doubtless, to the effect of fright and some unavoidable distress of the animal during the experiments.

The possibility that the cause of the increased acidity in the above reported experimental data was an accelerated oxidation with the accumulation of its accompanying products in the circulation led to the estimation of the blood reaction during and immediately following insomnia, where it was thought the prolonged metabolic processes taking place might give rise to a similar condition of the blood. But tests on a series of six rabbits, housed in a warm place, abundantly supplied with food and water but kept awake for 100 consecutive hours, were quite negative on this point.

Evidence of a similar sort is also obtained by the following experiments. In pithed animals immediately following the severing of the spinal cord there is a rapid rise of hydrogen-ion concentration, due undoubtedly to the decreased lung aëration and the consequent faulty elimination of carbon dioxide, for on the establishment of artificial respiration the normal reaction of the blood is promptly restored. When in such a pithed animal where a normal hydrogen-ion concentration was being uniformly maintained by artificial respiration, violent contractions of the skeletal muscles were produced by electrical stimulation of the cut end of the cord in communication with the musculature of the thorax, abdomen and extremities, no evidence of an increased acidity of the blood was found except when the animal was dying.

One other condition in which the blood showed an increased acidity was that of shock. Only two experiments were performed but as the results in both cases were very definite, they are now outlined.

I. Blood from dog's ear had a hydrogen-ion concentration of pH = 7.61. The animal was anaesthetized with ether, the abdomen opened and intestines manipulated for $1\frac{3}{4}$ hours at the end of which time the blood pressure was exceedingly low. Thirty minutes subsequent to this manipulation and 40 minutes after the ether was discontinued, the acidity of the blood was pH = 7.11; and 60 minutes follow-

ing intestinal manipulation and 70 minutes after the cessation of the administration of the ether, the acidity had risen to pH = 6.98.

II. Reaction of dog's blood at the beginning of the experiment was pH = 7.48. Shock evidenced by a marked fall in blood pressure was produced by a manipulation of the intestines. One hour after ether was discontinued, blood from the vena cava had a value of pH = 7.08. Blood pressure at this time was so low that sufficient blood for a reading could not be withdrawn from the femoral artery with a syringe. That the increased acidity was not due to imperfect elimination of ether was indicated by the return to consciousness of both animals in from 25 to 30 minutes after the anaesthetic was discontinued.

In addition to the conditions above reported, where increased acidity of the blood obtains, is appended lastly a very interesting observation where a diminution of the hydrogen-ion concentration was noted; namely, in the blood flowing from the adrenal gland. In order to obtain blood which would contain, as far as possible, the maximal concentration of adrenal secretion, it was found necessary to clamp the adrenal vein just before it joins the vena cava, on the one side, and before it passes over the adrenal gland on the other side. The portion of the vein, between the clamps therefore, lay directly over the gland and contained only blood from that organ. When this blood was withdrawn with a syringe and measured the value of the hydrogen-ion concentration was always from pH = 0.10 to pH = 0.12 lower than the blood taken from the immediately adjoining vena cava or from that part of the adrenal vein lying distal to clamp. While this difference is not large it is a very constant feature of adrenal blood. The assumption that this increased alkalinity is due to adrenalin is supported by the fact that the addition of adrenalin to blood serum, whose hydrogen-ion concentration is known, lowers its acidity and the diminution corresponds to the weight of adrenalin added when this is below the amount necessary for saturation. Thus if adding a definite amount of this substance, which as has been shown by Aldrich (10) and others to be a very strong base, causes a certain increase in the hydroxyl ion concentration, then when twice or four times that amount is added the increase in alkalinity is multiplied by two and four respectively. Since at no time was it possible, with the apparatus used, to obtain blood from the vena cava, in the immediate vicinity of the opening of the adrenal vein which was appreciably more alkaline than blood from any other part of the vena cava, the adrenalin apparently causes a measurable modification of the blood in a very limited area. Moreover, that the influence of the

adrenalin on the circulatory fluids is local in character is further evidenced by the fact that the removal of the gland caused no change whatever in the reaction of the blood until the animal was moribund. Although blood from the pancreas, liver and thyroid, as well as from the internal and external jugular veins was compared with blood from the vena cava of the same animal, no evidence of any variation in the hydrogen-ion concentration could be obtained. Further the removal of these organs singly or in combination caused no change in the reaction of the blood.

The data presented in this paper proves conclusively that a very marked increase in the hydrogen-ion concentration may occur under certain abnormal conditions, and that the existence of this high acidity of the blood is not incompatible with life. As to the cause of the phenomenon and regarding its significance, the authors feel the present data to be too limited to warrant much speculation. Concerning the first point, however, one or two suggestions may not be amiss. The immediate and intense response of the blood to emotional stimuli by a marked rise in the hydrogen-ion concentration with the corresponding alteration of the character of respiration, and the rapid disappearance of these on the removal of the exciting cause, indicate that under these circumstances the carbon dioxide is a major factor; it is obvious however that all increased acidity of the blood cannot be ascribed to this source. This explanation does not suffice for shock and anaesthesia, since in the former the carbon dioxide in the blood is markedly diminished as has been shown by Henderson (11), and in the latter the carbon dioxide in the blood is increased according to the researches of Buckmaster and Gardner (12). Indeed in such a composite fluid as blood, containing so many complex chemical compounds, it is quite conceivable as has been suggested by Robertson (13) that the amphoteric character of certain proteins must be of extreme importance in regulating the acidity of the blood.

SUMMARY

1. The hydrogen-ion concentration of the blood during certain emotional disturbances, such as fright in rabbits and dogs, and anger in cats, is markedly increased and at a temperature of 20°C., frequently reaches an acidity corresponding to pH = 7.00. This is probably due to increased carbon dioxide tension.

2. In anaesthesia caused by ether, chloroform and nitrous oxid the hydrogen-ion concentration may be increased to the same ex-

tent. The change in acidity begins when the inspired anaesthetic commences to react with the blood, and depends, approximately, on the degree of the anaesthesia. The restoration of the normal reaction of the blood is completed in 45 minutes after the administration of the drug is discontinued.

3. In two cases of shock the acidity of the blood was very much increased.

4. The blood flowing from the adrenal gland is always more alkaline than venous blood elsewhere in the body. This increased alkalinity is local, not extending to any appreciable extent beyond the immediate vicinity of the adrenal vein, and is due to the dissolved adrenalin which it contains.

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THE ORIGIN OF ANTITHROMBIN

GEORGE P. DENNY, M.D., Boston, GEORGE R. MINOT, M.D., Boston
From the Physiological Laboratory of the Johns Hopkins Medical School

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INTRODUCTION

It is a well-known fact that Witte's peptone when injected intravenously into dogs produces in their blood an anti-coagulating substance which causes blood withdrawn from such animals to remain fluid for long periods. The plasma from peptonized animals has the power to delay or inhibit the coagulation of normal blood in vitro.

Many workers have studied peptonized animals with a view of finding the origin of the anti-coagulating substance, among them being Delezenne (1), Nolf (2), Doyon (3), and Popielski (4). All agree that the liver is the most important site; in fact, with the exception of Popielski they feel it to be the only site. The last named author thinks the intestine and extremities play a large part.

Almost all experiments on the origin of antithrombin have been carried out by means of peptone injections and it has been found that in animals with the liver cut out of the circulation peptone is without effect on coagulation. The liver then seems essential to the production of the anti-coagulating substance which we shall call antithrombin according to Howell's theory (5).

Just in what way peptone acts on the liver is a disputed point but all workers agree that Witte's peptone itself has no action. It is some substance formed in the blood which stimulates the liver to produce antithrombin. Delezenne believed the destruction of leucocytes to play the whole part. Nolf's work does not substantiate this. Both Nolf and Popielski believe the liver endothelium produces antithrombin but this point is not as yet adequately proven. No attempt will be made in this paper to discuss the various theories of the method of peptone action but the above brief summary serves to indicate that the liver is the organ most concerned.

Up to the present time almost all determinations of antithrombin have been made by determining the effect of the peptone plasma on whole blood. This of course is a very rough method and serves only to show a great increase in antithrombin.

According to Howell's theory of coagulation antithrombin is a normal constituent of the blood. By its antagonizing action to prothrombin it prevents the latter from being converted to thrombin and tends to neutralize the action of any thrombin that may be formed. In other words it is the normal excess of antithrombin which prevents intravascular clotting. If antithrombin is normally present in the blood, and if, as previous workers have shown, it is formed in the liver, it would seem likely that in a normal animal blood taken from the hepatic vein would show a greater amount of antithrombin than blood from other parts of the body.

Doyon (6) has reviewed the numerous articles on the coagulation time and fibrinogen content of blood so taken and finds the results so greatly at variance that no conclusions are possible. He concluded from work of his own that such blood may coagulate more quickly than carotid blood. Nolf found that whole blood introduced into a living washed liver clotted normally.

We have taken up the study of blood after its normal passage through various organs and after stasis in the same organs.

TAPPING OFF OF ORGANS

In order to determine how the antithrombin content of the blood may vary in different parts of the body a series of six experiments were done, the results of which are tabulated below.

Method. A dog was etherized, after previous administration of morphia, and a tracheal cannula inserted for continued etherization and artificial respiration. Dissecting down on the jugular vein a specimen of blood was drawn from it as a control, ten to twenty minutes before a head thorax circulation was established. In all but Experiment II ligatures were then placed in the following order: around the inferior vena cava in the thorax close to the diaphragm, around the inferior cava directly below the liver, and around the aorta directly below the diaphragm. In Experiment II the order of the ligatures at the liver was reversed, first below and second above.

It took about ten minutes to place these ligatures after the vessels were exposed. The ligatures were allowed to remain until after the experiment was completed.

Specimens of blood were drawn as rapidly as possible after the stasis was produced, usually in the following order: first, from the portal vein, second, from the inferior vena cava below the lowest ligature, and third, from the hepatic vein. In Experiments III and V the order was, the inferior cava, the hepatic vein and the portal vein. It was about *ten* minutes from the time the last ligature was tied until the last of the above samples of blood was obtained. The antithrombin determinations of these specimens are recorded in the table as "before stasis." To be sure, a few minutes of stasis of the blood had occurred before these samples were obtained, but they represent blood before prolonged stasis had taken place. After the blood below the diaphragm had remained stagnant and a head thorax circulation had existed for varying lengths of time, 30–70 minutes, as recorded in each instance in the table, other specimens of blood were drawn from the same regions for comparison.

Samples of blood were drawn from the splenic and renal veins in three experiments before the ligatures creating a head thorax circulation were tied; then the arteries and veins of these organs were clamped and after an interval of stasis blood was again drawn to be compared with other specimens.

The specimens of blood to be examined were drawn with a Luer syringe previously rinsed with salt solution. A definite volume of this blood was put into a tube with a constant amount of 1 per cent sodium oxalate in 0.9 per cent sodium chloride solution and well mixed, centrifuged for 20 minutes, and the plasma pipetted off, heated to 60°C. to precipitate the fibrinogen and destroy prothrombin, filtered and examined for antithrombin by the method described by Howell (7) and discussed by Minot and Denny (8).

In brief the test used was as follows: 1 drop of the antithrombin solution (after heating to 60°C. and filtering) from each specimen was mixed in a tube with the varying amounts of a solution of thrombin. After these two substances had been in contact for a given interval, a constant amount of a fibrinogen solution was added to each tube so that the clots formed in a period of time suitable to distinguish differences. The time taken for clotting was upon the first appearance of a clot, that which clotted first having the least antithrombin and that last the most. At least four suitable series of determinations on the different specimens were made. To compare the specimens of any single experiment, the time that their antithrombin allowed thrombin and fibrinogen to clot was sufficient.

But in order to compare those of different experiments time could not be satisfactorily used, owing to differences in the reagents, so that antithrombin is expressed as the antithrombin factor (8). The average time of clotting of a series of suitable determinations of a specimen was divided by a similar figure obtained for a control. The control in these experiments was the specimen from the jugular vein before any stasis was produced and its factor was taken as unity.

By referring to the table it will be noticed that in the six different experiments the antithrombin in the venous blood of the jugular or superior vena cava before and after a head thorax circulation remained about the same.

The two observations of splenic blood before stasis showed less antithrombin than the jugular blood. The three specimens of splenic vein blood after stasis all gave a plasma in which some definite hemolysis had taken place. It has been shown that large amounts of fatty acids occur in the spleen and this may be the reason that the blood standing in the spleen is hemolyzed. Except for one specimen of renal plasma, none of the other specimens showed any hemolysis. It was felt that the hemolysis in this renal specimen might have been due to the fact that the syringe contained water and was not washed out with salt solution before the blood was drawn.

Antithrombin, being "neutralized" by thromboplastin, cannot be determined satisfactorily in a plasma in which hemolysis is apparent, so that the figues for antithrombin after stasis of blood in the spleen are probably lower than they should have been owing to the presence of thromboplastin liberated from the hemolyzed corpuscles. The two observations of antithrombin in renal vein blood before stasis were similar to the control with a slight tendency to rise after stasis.

Portal blood before prolonged stasis was studied in five experiments; in four it contained less antithrombin than the control, in one a greater amount. In the three observations after an interval of stasis from 32–60 minutes there was a slight rise.

Inferior vena cava blood (taken from below the lowest ligature) in the four experiments in which it was studied showed less antithrombin than the control, with a definite rise after an interval of 32 to 48 minutes of stasis in three instances, and essentially no change in the fourth.

Hepatic vein blood before prolonged stasis showed an antithrombin content essentially like the control blood. Just before the specimen to be tested after stasis of 30 to 70 minutes was obtained, varying amounts of blood were drawn off from the vein and discarded so as to

obtain blood that had been in actual contact with the liver cells. Owing to the short length of the hepatic vein it was easy to draw blood from the cava that had stood between the clamps above and below the liver instead of obtaining hepatic vein blood. To obviate this difficulty in the last three experiments just before the hepatic blood after prolonged stasis was drawn, a clamp was placed across the cava above the liver, just below the point where the hepatic vein enters it.

After prolonged stasis of blood in the liver there was in the last four experiments a very marked rise in the antithrombin, a much greater amount than in blood from other sources. In the first experiment there was a slight rise and in the second a fall. Why there was not a greater rise in Experiment 1 and a fall in Experiment 2 is not clear, but it is possible that the blood obtained had not been stagnating deep in the liver tissue.

Not only does the antithrombin show a distinct increase after stasis of blood in the liver, but the clot formed by the thrombin and fibrinogen in the presence of the antithrombin from this source was of a weak character such as occurs when one has increased antithrombin.

The portal blood and the blood from the inferior cava and spleen, obtained before stasis, had less antithrombin in most instances than that from the jugular. The renal blood on the contrary had about an equal amount, as did the hepatic. Blood taken after stasis in any organ showed in most instances a slight rise in antithrombin. This was particularly evident in the inferior cava below the lowest ligature, but in no case was found the great rise observed after stasis in the liver.

In Experiment VI the time of clotting of the recalcified oxalated plasma was determined. Precautions were taken to find the optimum amount of calcium for this clotting and the time obtained designated as the prothrombin time. It constitutes a test of the relative efficacy of the prothrombin present. To a series of tubes each with 5 drops of plasma from each specimen 2, 3, 4 and 5 drops of 0.5 per cent $CaCl_2$ solution were added and the time of clotting noted. The tubes with 3 drops of calcium were the first to clot in all instances, i.e., had the optimum amount of calcium. The shortened time occurring with plasma from the splenic blood after stasis in this organ, is undoubtedly due to the hemolysis and the consequent liberation of thromboplastin as we have frequently noticed on other occasions.

Prothrombin times are as follows:

Jugular vein before stasis 5 minutes.

Jugular vein after stasis 5 minutes.

Splenic vein after stasis 3 minutes.
Renal vein before stasis 5 minutes.
Renal vein after stasis 5 minutes.
Portal vein before stasis 5 minutes.
Portal vein after stasis 5 minutes.
Hepatic vein before stasis 5 minutes.
Hepatic vein after stasis 35 minutes.

A striking delay in the prothrombin time occurred with the hepatic blood after stasis. This delay is probably wholly due to the increased antithrombin content, although possibly there was also a decreased amount of prothrombin.

Examination of arterial and venous blood taken from the heart has not shown any difference in either antithrombin or prothrombin content.

PERFUSION OF LIVER

Perfusion of the liver with mixtures of blood and peptone, plasma and peptone, etc., has been shown by Delezenne (1), Nolf (2) and others to produce antithrombin. Both of these authors have perfused the liver with defibrinated blood alone and Delezenne came to the conclusion that such blood acquired a very slight anticoagulating power. Nolf also obtained negative or doubtful results.

Doyon (9), by attaching the carotid artery of one dog to the portal vein of another which had been bled to death found that on emergence from the inferior cava the blood was slow to coagulate and retarded the clotting of normal blood. Positive results were obtained only with young, fasting dogs. With the exception of the above work we have been unable to find in the literature any successful demonstration of a formation of antithrombin by perfusion of the liver with a normal circulating medium without drugs added to the perfusing material.

We have perfused the liver with defibrinated blood in the following manner:

Method. A large dog was bled to death while under ether and the blood defibrinated. Part of this was diluted 1 to 2 with normal salt solution and used for irrigation and the rest was used to perfuse the liver of a second dog.

Dog II was given morphia and ether, the abdomen opened and the portal vein exposed. A large cannula was placed in the portal vein and the inferior cava quickly clamped above its tributaries, close to the liver. The hepatic artery was also clamped at this time.

	EXTERNAL JUGULAR OR SUPERIOR VENA CAVA			SPLENIC VEIN			RENAL VEIN			PORTAL VEIN			INFERIOR VENA CAVA BELOW LOWEST LIGATURE			HEPATIC VEIN		
	Antithrombin before starting experiment	Antithrombin at end of experiment	Duration of the head thorax circulation	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Antithrombin before stasis	Antithrombin after stasis	Duration of stasis	Amount of blood withdrawn before specimen was taken cc.
Experiment I	1 0	0 93 0 85	8' 40'		0 64	5'		1 07	3'	0 66	1 3		0 73			1 07	1 22	30'	29
Experiment II	1 0	1 16	60'							0 77			0 71		40'	1 00	0 83	50'	15
Experiment III	1 0	1 0	30'							0 92			0 77	1 23	32'	1 07	1 80 1 76	35' 37'	18 25
Experiment IV	1 0	1 0	35'	0 85	0 80	25'	1 0	1 2	30'				0 78	1 30	40'	1 07	1 85 2 02	43' 45'	29 39
Experiment V	1 0	1 0	50'							0 68	1 09	35'	0 68	1 06	38'		2 18 1 70 2 22	40' 42' 44'	12 29 30
Experiment VI	1 0	1 2	70'	0 80	0 80	30'	1 2	1 3	40'	0 9	1 20	60'				1 00	3 00 3 80	65' 70'	24 39

While one operator was doing this the other had opened the chest and introduced a cannula into the inferior cava just above the liver and washing with diluted blood was immediately started through the portal vein. As soon as the washing fluid had been run through perfusion was started with defibrinated blood. The time during which the liver had no circulation varied from 4 to 7 minutes.

The apparatus used for perfusing was in most cases very simple. It consisted of a large tank filled with water. In this was placed the reservoir for the perfusing and washing fluids from which tubes lead to the inflow cannula in the portal vein. The flow was by gravity. A thermometer was introduced at a point just before the entrance into the liver and the perfusing fluid was kept at about 38°C. by regulation of the temperature in the large water bath.

The outflow was caught in a container kept at about 38° by means of a water bath. The outflow was whipped, when defibrinated blood was used as a perfusate, since it was found that the first specimens coming through after washing showed some tendency to clot. Defibrination was continued only during the first part of the experiment and was necessary probably because the liver had not been washed entirely free from blood. In some experiments oxygen was bubbled into the outflow container and in one an artificial lung devised by Dr. Hooker was used. Oxygenation seemed to make little or no difference since those experiments in which no attempt was made to oxygenate gave similar results. The outflow was poured back by hand into the reservoir above and thus allowed to circulate many times.

Perfusion was carried on under a pressure of 90 to 120 mm. of mercury. Although there was a certain amount of loss in the amount of the perfusing liquid the latter was not concentrated enough to account for any significant increase in antithrombin content. The method, however, has the objection that the system was not closed.

Specimens were taken of the perfusate before perfusion was started and at intervals during perfusion. These were taken in 5 cc. lots and mixed with 0.7 cc. of 1 per cent oxalate in normal saline. After centrifuging, antithrombin tests were done on the serum by the method of Howell previously described.

Four experiments were done.

Experiment I. Perfusion with Ringer's solution.

Dog—Morphia and ether. Cannulas placed as described. Liver washed with 3 liters of Ringer's solution until outflow was clear.

Perfusion then started with about 200 cc. of Ringer's solution. This was clear at the start but quickly became bloody. Whole system cleaned out and started again with fresh Ringer's. Perfused in first case for about one hour and in the second for one and a half hours. The latter became bloody and a third perfusion was done with fresh Ringer's after washing. After one hour and forty-five minutes perfusion was again bloody. The specimens taken during the last perfusion showed a heavy precipitate on heating to 60° which was not fibrinogen and was evidently protein from the dying liver. None of the seven specimens taken during the perfusion showed any more antithrombin than might be explained by the blood which had been picked up during the repeated passage through the liver.

Experiment II. Perfusion with defibrinated blood.

Preparation as described.

Liver washed out with 100 cc. defibrinated blood plus 200 cc. normal saline. Perfusion started with 570 cc. defibrinated blood after a control specimen had been taken. This is labeled I in the table. Specimens taken 30, 40, 50, 58 and 64 minutes after the start of perfusion are labeled II, III, IV, V, and VI.

All specimens centrifuged at high speed for 20 minutes, serum pipetted off, heated to 60° and filtered. On heating none showed any precipitate.

One drop of the heated and filtered serum (antithrombin) was added to the thrombin in varying amounts and after an interval of 15 minutes 10 drops of fibrinogen were added.

Antithrombin determination

THROMBIN	I	II	III	IV	V	VI	SPECIMEN NUMBER
4 gtt.	18′	30′	37′	41′	46′	48′	Figures represent time in minutes which it took for clot to form after addition of fibrinogen.
5 gtt.	12′	22′	22′	27′	38′	40′	

The two series correspond well and show a very marked gradual increase in antithrombic power.

Experiment III. Perfusion with defibrinated blood.

Large dog bled to death and cannulas inserted into the portal vein and inferior cava of another dog in the usual way. Liver washed with 100 cc. defibrinated blood plus 200 cc. normal salt solution.

Perfusion started after control specimen had been taken and 20 minutes later Specimen II was obtained.

Just after this was taken a rather large amount of water was spilled accidentally into the outflow jar. This of course produced great hemolysis as well as dilution in the circulating blood and the effect in lowering antithrombin will be seen in the table.

Specimens taken 65, 95 and 108 minutes after start of perfusion are labeled III, IV and V in the table.

On centrifuging, those specimens taken after the accident were much more hemolyzed than those before it. Antithrombin tests were run in the usual way.

Antithrombin determination

THROMBIN	I	II	III	IV	V	SPECIMEN NUMBER
5 gtt.	21′	21′	7′	14′	16′	Figures represent time in minutes which it took a clot to form after addition of fibrinogen.
6 gtt.	10′	11′	7′	10′	12′	

No. III represents the first specimen taken after spilling water into the blood. Nos. IV and V show a gradual increase in antithrombin content, although the antithrombin never reached the concentration that it had at the beginning.

Experiment IV. Perfusion with defibrinated blood.

This experiment was carried on in the usual way except that the outflow from the liver was connected with an artificial lung devised by Dr. Hooker. This ensured thorough oxygenation of the blood. The liver was washed with mixture of 300 cc. normal salt solution and 150 cc. defibrinated blood. Perfusion started after taking control, Specimen I. Specimens II, III, IV and V taken 30, 59, 70 and 83 minutes respectively after the start of perfusion. After Specimen V was taken 1.5 gm. peptone in solution was added to the perfusing material (about 400 cc.). Specimens taken 15, 24 and 34 minutes after addition of peptone are labeled VI, VII, and VIII in the table.

On centrifuging, all specimens showed hemolysis as usual, the degree increasing somewhat as the experiment went on.

Antithrombin determination

THROMBIN	I	II	III	IV	V	PEPTONE ADDED	VI	VII	VIII	SPECIMEN NUMBER
5 gtt.	10′	13′	13′	19′	No clot in 30′		No clot in 30′			
6 gtt.	8′	8′	8′	9′	Very poor clot 15′		Poor clot in 21′	26′	40′	

From this table it will be seen that antithrombin was not acquired by the perfusing fluid for about 1 hour, Specimen IV being the first to show any change. Specimen V shows a well marked increase. The specimens taken after addition of peptone show a still further increase in antithrombin content but not to the extent which one would expect

in a fresh liver perfused with a blood and peptone mixture. Probably much of the antithrombin had been taken up from the liver in this case before the peptone was added to the perfusate.

These experiments show that the frequent passage of defibrinated blood through the liver results in a gradual increase is the antithrombin content of the perfusate.

Specimens of defibrinated blood were obtained from perfusions of the spleen, head and hind leg done by other workers in this laboratory, and although in some of these cases the perfusions were carried on much longer than in our experiments with the liver, we were unable to demonstrate any marked increase in antithrombin; in fact, usually the amount was decreased. Three perfusions of the head showed in each instance a very slight increase of antithrombin.

The decrease in antithrombin was probably due to hemolysis and deserves a word in connection with our experiments.

The centrifuged serum from defibrinated blood always shows a certain amount of hemolysis. In perfusing for any length of time the amount of this hemolysis increases distinctly up to a certain point. Since hemolysis liberates an active thromboplastic substance some of the antithrombin in the perfused blood must have been neutralized and therefore the increase in antithrombin which we actually found after perfusion of the liver probably did not represent the total increase obtained. If we could have done away with this factor of hemolysis our results would probably have been more striking.

ATTEMPTS AT STIMULATION OF LIVER

Having shown that stasis in the liver causes an increase in antithrombin and that perfusion with defibrinated blood likewise causes an increase we next attempted to stimulate the liver in various ways.

Doyon (10) has attempted this also and claims success as a result of intravenous injection of bile and bile salts. He found that bile salts injected intravenously could cause an incoagulable blood, and that doses which were ineffective in the general circulation were capable of delaying coagulation when injected into the mesenteric veins. He did not show that the long coagulation time was due to an increased antithrombin.

The substances which we have used were all injected into the mesenteric veins, and all the dogs used were starved 24 hours or longer. These experiments will not be given in detail because they were entirely negative.

Experiment 1. Effect of bile salt. Dog, weight 7 kg.

9.5 cc. of 10 per cent solution of sodium glycocholate was injected into the mesenteric vein. In 10 minutes the coagulation time had gone from 40 minutes (normal) to one hour and twenty minutes and remained in this vicinity for three hours.

All specimens taken after injection showed a very marked hemolysis and the antithrombin was uniformly reduced as compared with the control specimen. Prothrombin not changed to any marked degree. We felt that larger doses might cause greater delay in coagulation time but that the increasing hemolysis would make it impossible to say if it were due to an increased antithrombin.

Experiment 2. Effect of bile by mouth.

A dog, weight 8 kg., having had control specimen of blood taken, was given 16 gm. of dried ox bile in 200 cc. water by stomach tube. Allowed to come out of ether. Vomited a little (40-50 cc.).

Two hours and fifteen minutes after giving bile another specimen of blood was taken. 17 gm. of ox bile in 150 cc. water then given by stomach tube. Specimens taken one and two hours later showed no hemolysis, no change in coagulation time and no change in prothrombin and antithrombin content.

Experiment 3. Effect of secretin.

Dog, weight 7 kg. Morphia and ether.

10 cc. of a secretin solution prepared according to Bayliss and Starling (11) injected into the mesenteric vein. Specimens taken 10 minutes after, showed coagulation time of 56 minutes against the control time of 28 minutes—20 minutes after injection coagulation time was 29 minutes. 9 cc. more injected and specimens taken 16 and 24 minutes later, showed a coagulation time of 41 and 55 minutes respectively.

Large amounts of secretin introduced into the mesenteric veins with a burette failed to further lengthen the coagulation time.

No pancreatic fistula having been made, it is not certain that the secretin used was active. There was a slight lengthening of the coagulation time after the first two injections but on the whole the experiment must be considered negative. There was no change in the antithrombin content of the numerous specimens taken after the various injections.

Experiment 4. Effect of nerve stimulation.

Dog, weight 8 kg. Morphia and ether. Control specimens taken for coagulation time and antithrombin. Abdomen opened and coeliac plexus exposed. Electrode passed under the ganglion and stimulation applied with a rather strong current from an induction coil. Duration of stimulation 20 seconds with an interval between of 40 seconds. The procedure was continued for one hour, specimens being taken at 15, 35 and 50 minutes from the start of stimulation.

These specimens showed no change in coagulation time or antithrombin content. The hepatic branches of the plexus were next isolated and stimulated with the result that no change was obtained either in coagulation time or antithrombin.

Experiment 5. Effect of injection of thrombin.

Dog, weight 8 kg. Morphia and ether.

Injection of 0.4 gm. of pure thrombin in which there was some salt (this solution was very active in clotting fibrinogen) into the mesenteric vein. In a specimen taken 12 minutes after injection there was lengthening of coagulation time

from 30 minutes (normal) to 43 minutes and an increase in antithrombin from 10 minutes to 17 minutes.

Davis (12) found an increase in antithrombin by injecting thrombin in very large amounts into the general circulation. Here we see a small amount into the mesenteric vein giving a slight but definite effect.

PHOSPHORUS POISONING

Antithrombin as Influenced by Phosphorus Poisoning in Dogs

If the liver is the site of antithrombin formation it would seem probable that a liver destroying substance such as phosphorus would lower the antithrombin content of the blood. It has been shown by many Jacoby (1900); Loeb (1903); Morawitz (1905); Doyon (1906); Nolf (1908); and Whipple and Hurwitz (13) that the fibrinogen of the blood in phosphorus poisoning is greatly diminished or almost absent.

We have studied the blood of five dogs with varying degrees of phosphorus poisoning, all of which showed extensive liver destruction at autopsy. The specimens of blood were kindly given us by Drs. Goodpasture and Marshall, to whom we wish to express our thanks.

Antithrombin determination

	PHOSPHORUS DOG	NORMAL DOG	ANTITHROMBIN FACTORS
Dog I	9′	14′	0.64
" II	8′	15′	0.53
" III	11′	25′	0.44
" IV	2′	16′	0.12
" V	2′	13′	0.15

Figures represent time of formation of a clot in the usual antithrombin test. It will be remembered that the normal antithrombin factor is approximately 1.

Dog IV was the most severely poisoned and shows the greatest diminution in antithrombin; in fact, almost a complete absence of any anticoagulating power. All specimens showed by rough tests a diminution in fibrinogen.

These results tempt speculation as to what part of the liver is concerned in the formation of antithrombin. It will be remembered that Nolf and Popielski are inclined to think that the endothelium in the liver is responsible.

While it cannot be stated that phosphorus does not injure the endothelium of the liver there can be no doubt of its destructive action upon the liver cells and we suggest from our results that antithrombin may very likely be a true liver cell product. However, further work on this subject, with careful histological study, must be done in order definitely to demonstrate this point.

Numerous clinical cases of liver disease which we have studied have shown no constant variation in antithrombin content of the blood. A few with low fibrinogen have shown a low antithrombin. Some cases having a normal antithrombin showed very extensive destruction of liver substance at autopsy but it is a fairly well recognized fact that only small amounts of active liver tissue are necessary to carry on the functions of the liver.

CONCLUSIONS

1. Antithrombin is formed in the liver.

2. Venous blood taken from the liver, spleen, kidneys and intestines shows no appreciable difference from jugular vein blood in antithrombin content.

3. When stasis is produced in these organs the liver blood alone shows a definite increase in antithrombin.

4. Perfusion of the liver with defibrinated blood causes an increase of antithrombin in the perfusate.

5. Perfusion of the head, spleen and hind leg shows no marked increase and generally a decrease in antithrombin content.

6. Attempts to stimulate the liver to production of antithrombin by means of bile, bile salts, secretin and electrical stimulation were negative. Injection of small amounts of thrombin into the portal circulation caused a slight rise in antithrombin.

7. Dogs with phosphorus poisoning and liver destruction show a very marked decrease in antithrombin content of the blood as well as diminished fibrinogen.

We wish to express our gratitude to Professor Howell for invaluable advice and suggestions and to thank Dr. D. R. Hooker for material from his experiments and for help with our perfusion work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXV. A Note on the Chemistry of Normal Human Gastric Juice

A. J. CARLSON

Assisted in Part of the Experiments by H. Hager and M. P. Rogers

From the Hull Physiological Laboratory of the University of Chicago

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The gastric juice employed in this work was obtained from Mr. V., our gastric fistula case with complete cicatricial stenosis of the oesophagus of 20 years' standing. The gastric juice is therefore at no time mixed with saliva. The appetite juice is thus secreted during the mastication of the food and some data relative to the secretion of appetite and hunger gastric juice in Mr. V., as a type of normal adults, have already been reported (1). A few samples of appetite gastric juice was secured from a second gastric fistula case with partial cicatricial stenosis of the oesophagus. Samples of appetite and of hunger gastric juice were also secured from one of the authors (A. J. C.) by a method that will be referred to later. A few controls are also noted on dog's gastric juice, that is, the secretion from the accessory stomach pouch prepared according to Pawlow. Most of the present work refers to appetite gastric juice. A few determinations were made on samples of the hunger gastric juice. The reader is referred to our previous report for an account of the continuous or hunger gastric secretion.

I. *The solids of the gastric juice.* 100 cc. of six different lots of appetite gastric juice of Mr. V. were evaporated on water bath and then dried to constant weight in an oven at 112° C. The incineration was made at dull red heat so as not to drive off the potassium chloride, which according to Rosemann is more abundant in gastric juice than the sodium chloride (2). The results are given in Table I. On three of these lots of appetite juice the specific gravity, the freezing point, and the total acidity (phenolthalein) was also determined. The one determination on hunger gastric juice represented many separate collections of juice, as no juice was included in this lot if it was secreted by

the empty stomach at a greater rate than 3–4 cc. per hour. For the purposes of comparison the analyses of two lots of the juice or fluid found in the stomach free from food one hour after putting 200 cc. of water into the stomach are also given in Table I. It will be seen from Table I that the total solids of the appetite juice vary from 0.48 gr. to 0. 58 gr. per 100 cc., of which 0.34 gr. to 0.47 gr. is organic, and 0.11 gr. to 0.14 gr. inorganic material. The hydrochloric acid is, of course, expelled in the process of evaporation and drying of the gastric juice residue.

The hunger gastric juice of Mr. V. is distinctly higher than the appetite juice in total, and in organic solids. It appears also to be higher than the average appetite juice in inorganic solids.

The gastric juice or fluid in the stomach free from food one hour after ingestion of 200 cc. of water is distinctly more dilute than the

TABLE I

The composition of pure human gastric juice. The solids of appetite gastric juice, hunger juice, and content of "empty" stomach per 100 cc. of juice.

	APPETITE GASTRIC JUICE						HUNGER GASTRIC JUICE	CONTENTS OF THE "EMPTY" STOMACH	
	I	II	III	IV	V	VI		I	II
Specific gravity	1007	1009	1006		1008			1004	1006
Δ	−0 550	−0 580	−0 530					−0 31	−0 45
Acidity (total)	0 45	0 48	0 42				0 34	0 22	0 33
Total solids	0 557 gr.	0 606 gr.	0 480 gr.	0 571 gr.	0 580	0 544	0 667 gr.	0 360 gr.	0 450 gr.
Organic	0 420 gr.	0 466 gr.	0 341	0 452 gr.	0 470	0 430	0 521 gr.	0 260 gr.	0 350 gr.
Inorganic	0 137 gr.	0 140 gr.	0 139	0 119	0 110 gr.	0 114 gr.	0 146 gr.	0 100 gr.	0 100 gr.

appetite juice, although it may approach the concentration of the latter in cases where the rate of the continuous secretion is considerable (Lot II).

The above figures on Mr. V.'s appetite gastric juice are slightly higher than those given by Sommerfeld (3) for the gastric juice from a ten-year-old girl, namely, 0.40 gr. to 0.47 gr. Sixty years ago Schmidt (4) reported on the gastric juice of a human gastric fistula case, the oesophagus being partly patent. He found total solids 0.58 per cent, of which 0.32 per cent was organic, and 0.26 per cent inorganic. But Schmidt did not work with pure gastric juice This is evident from his method of obtaining the juice, as well as from the fact that the acidity of the juice was only 0.20 per cent, which is less half that of normal human gastric juice. The figure of 0.26 per cent for inorganic solids is probably also too high Albu (5) reports one experiment o.1

a patient with hypersecretion finding the percentage of solids only 0.24 gr. practically all of which (0.23 gr.) was inorganic salts. Albu also reports one determination on normal human gastric juice (pure appetite juice) in which the inorganic solids were 0.18 per cent; the organic solids are not given.

Our results on Mr. V. agree closely with most of those reported for the gastric juice of dogs, as the following figures will show:

Observer	*Total Solids per cent*	*Organic per cent*	*Inorganic per cent*
Pawlow and Schoumow-Simanowksi (6)	0.47		
Konovaloff (7)	0.48		
Schoumow-Simanowksi (8)	0.528	0.393	0.135
Rosemann (2)	0.427	0.294	0.132
Nencki and Sieber (9)	0.306		

The total concentration of organic and inorganic substances is therefore about the same in the normal gastric juice (appetite juice) of man and dog. The very low solids (in one case only 0.16 per cent) reported by Nencki and Sieber are clearly exceptional and must be due to some unusual condition of their animal.

II. *The specific gravity of the gastric juice.* The specific gravity of Mr. V.'s appetite gastric juice varies between 1006 and 1009 with an average of 1007. This is the average of twenty tests on an equal number of gastric juice samples. It will thus be seen that 1009 is an exceptional concentration.

The specific gravity of the hunger juice is certainly higher than that of the appetite juice, since the total solids are higher, but no actual measurements were made on the hunger juice. The specific gravity of the fluid or juice found in the empty stomach thirty to sixty minutes after ingestion of water varies from 1003 to 1006, with an average of 1004.

The specific gravity of the appetite gastric juice of the dog, as reported by Schoumow-Simanowksi, Konowaloff, Friedenthal (10), and Rosemann varies from 1002 to 1007, with an average of 1004. This low average figure is probably due to the fact that in most of the experiments the gastric juice was collected for several hours after only a few minutes sham feeding. There is evidence that the percentage of solids in the gastric juice is greatest during the first hours of appetite or digestion secretion. The concentration of the dog's appetite gastric juice during the first twenty minutes of secretion will in all probability be found identical with that of man for the same period.

III. *The osmotic concentration of the gastric juice.* Our data on the osmotic concentration of the gastric juice are brought together in Table II. The appetite juice lowers the freezing point, -0.55° C. to -0.62° C.; the hunger juice from -0.47° C. to -0.52° C.; the fluid of the empty stomach -0.21° C. to -0.41° C. The juice found in the empty stomach an hour after ingestion of water exhibits the

TABLE II

The osmic concentration of normal human gastric juice (Mr. V.) The total acidity of the samples of gastric juice were determined with phenothalein as the indicator

FLUID IN "EMPTY" STOMACH		HUNGER JUICE		APPETITE JUICE	
Total acidity	Δ	Total acidity	Δ	Total acidity	Δ
per cent		*per cent*		*per cent*	
0.33	−0.38	0.40	−0.51	0.53	−0.58
0.26	−0.33	0.39	−0.52	0.52	−0.61
0.35	−0.42	0.36		0.45	−0.57
0.26	−0.30	0.35	−0.51	0.53	−0.60
0.30	−0.39	0.32	−0.50	0.52	−0.61
0.27	−0.38	0.36	−0.53	0.47	−0.59
0.18	−0.25	0.29	−0.49	0.42	−0.55
0.33	−0.38	0.28	−0.45	0.47	−0.60
0.17	−0.21	0.30	−0.47	0.49	−0.57
0.21	−0.27	0.41	−0.52	0.53	−0.60
0.17	−0.25	0.36	−0.49	0.45	−0.59
0.25	−0.32	0.41	−0.53	0.50	−0.61
0.26	−0.33	0.40	−0.52	0.49	−0.60
0.27	−0.37	0.32	−0.50	0.51	−0.57
0.18	−0.28	0.25	−0.47	0.46	−0.62
	−0.33			0.51	−0.60
	−0.37			0.51	−0.60
				0.47	−0.57
				0.48	−0.55
				0.49	−0.59
				*	−0.62
					−0.62

greatest fluctuations in osmotic pressure, the appetite and the hunger juice being very constant. The hunger juice has, on the whole, a lower osmotic concentration than the appetite juice. The total acidity of the hunger juice is also uniformly lower.

The above figures for the appetite gastric juice of Mr. V. are practically identical with those reported on the pure gastric juice of other

human fistula cases. Sommerfeld (in a ten-year-old girl) found the freezing point to vary from $-0.47°$ to $-0.65°$ C.; Kaznelson (11) (25-year-old girl) reports a variation from $-0.46°$ to $-0.54°$. Umber (12) reports two tests on the gastric juice (pure) of a 59-year-old man with cancer, finding a variation of $-0.15°$ C. to $-0.82°$ C. Assuming that Umber's determinations are correct, the gastric juice of this cancer patient was clearly not normal. We question whether the normal stomach can secrete a juice with an osmotic concentration so much greater than the blood as the figure $-0.82°$ C. demands. The reader will note that the figures of Sommerfeld and Kaznelson, as well as our own for Mr. V., indicate an osmotic pressure of the appetite gastric juice not far below or above that of the human blood. According to Bickel (13) the gastric juice (10-year-old child) is always hypotonic to the blood. Lehmann (15) concludes that the osmotic pressure of normal gastric juice (gastric content) is usually less than $-0.50°$ C. and that a concentration above this figure indicates hyperacidity or other pathological conditions. This view is obviously untenable.

The osmotic concentration of the dog's appetite gastric juice appears to be practically identical with that of man. Sasaki (14) reports a variation from $-0.51°$ to $-0.60°$; Rosemann gives somewhat higher figures as $-0.56°$ C. to $-0.64°$ C. On the other hand, Bickel (13) reports extraordinary fluctuations in osmotic concentrations of dogs gastric juice (Pawlow pouch) or $-0.52°$ to $-1.21°$ C. We question whether the normal stomach can secrete a juice of the osmotic concentration $-1.21°$ C., that is, twice that of the blood.

IV. *The nitrogen of the gastric juice.* 1. The total nitrogen was determined by the method of Kjeldahl on nine different lots of appetite gastric juice of Mr. V., with the following results:

Appetite gastric juice	*Nitrogen per 100 cc. gr.*
Lot I	0.074
Lot II	0.051
Lot III	0.054
Lot IV	0.057
Lot V	0.054
Lot VI	0.051
Lot VII	0.075
Lot VIII	0.057
Lot IX	0.071
Average	0.060

The average of all our determinations is 0.060 gr. nitrogen per 100 cc. appetite juice. The total nitrogen of the hunger juice was not determined. If all the nitrogen is in the form of proteins, and if we accept the figures of Nencki and Sieber, and of Pekelharing (16) namely, that the nitrogen constitutes 14.39 per cent of the proteins of the gastric juice, the appetite gastric juice of man would contain on the average nearly 0.42 gr. protein per 100 cc. This is practically all the total organic solids in the appetite juice (Table I). It is therefore probable that some of the nitrogen in the gastric juice is present in non-protein combinations, such as ammonia, amino acids, and sulphocyanic acid.

The literature does not, to our knowledge, contain any data on the total nitrogen of pure human gastric juice. Rosemann reports nitrogen determinations on two lots of dog's appetite gastric juice, finding 0.035 gr. and 0.054 gr. per 100 cc. respectively. The reader will note that these figures are considerably lower than those on the appetite juice of Mr. V.

2. *The ammonia of the gastric juice.* The ammonia of the fresh gastric juice was determined by a combination of Folin's aeration and the Nessler colorimetric methods, using 1 to 5 cc. of the juice. It appears that the ammonia cannot be determined by the Nessler reagent directly in pure gastric juice, as parallel tests on the same samples of gastric juice yielded higher figures by aeration and Nessler than by Nessler direct (using 1 cc. of the juice).

Our data, summarized in Table III, go to show that

1. Ammonia in the amounts of 2–3 mgr. per 100 cc. is a constant constituent of pure gastric juice of man and dog.

2. The ammonia appears to be slightly more concentrated in the continuous secretion or hunger juice than in the appetite juice.

3. The ammonia appears to be greatly increased in gastric ulcers (dog).

Rosenheim (17) and Strauss (18) reported small amounts of ammonia in the gastric content of man. Zunz (19), working with the gastric content (test meals) on normal persons and on persons with various disorders of the alimentary tract, also reports the presence of ammonia. In the normal individuals the ammonia of the test meal contents varied from 0.7 to 5.0 mgr. per 100 cc. In cancers of the stomach the ammonia in the test meal content is greatly increased, according to Zunz's figures. Zunz concludes there is no relation between the state of the gastric digestion or the acidity of the gastric

content and the quantity of ammonia in the gastric content. Nevertheless, the test meal introduces factors (bacterial action, saliva, etc.) not present in pure gastric juice. Sommerfeld (13), working with pure gastric juice of a ten-year-old girl with complete stricture of the oesophagus, states that gastric juice contains no ammonia. Nencki, Pawlow, Zaleski (20) and Salaskin (21) reported 4 to 4.5 mgr. ammonia per 100 cc. pure gastric juice of the dog. Rosemann (2) reports

TABLE III

The ammonia in gastric juice

INDIVIDUAL	MATERIAL	NO. OF OBSERVATIONS	NH_3 IN MGR. PER 100 CC. GASTRIC JUICE		
			Maximum	Minimum	Average
Mr. V.	Appetite juice	30	5.5	2.0	3.0
	Hunger juice	10	5.6	3.0	4.0
Mr. E*	Appetite juice	8	3.0	1.5	2.0
Mr. C.†	Appetite juice	5	8.5	7.5	8.0
	Hunger juice	3	10.0	9.5	9.8
6 normal dogs with Pawlow pouches	Appetite juice	20	4.5	1.5	2.5
	Digestion juice	24	4.5	1.5	2.5
Dog‡ Pawlow pouch and ulcer in pouch	Continuous secretion	5	25.0	10.0	18.0

* Mr. E. is a second gastric fistula case, a man age 24, partial cicatricial stenosis of oesophagus from drinking lye. Gastrostomy of five months' standing. The man is in good general health, and shows no gastric disturbance.

† Mr. C. noticed increased hunger pains, and at times slight epigastric pain for a period of several months when these tests were made. There was no variation in the diet.

‡ This dog had an experimental ulcer in the stomach pouch produced by intravenous injection of a strain of streptococci isolated from a gastric ulcer in man by Dr. C. E. Rosenow. This dog was used in another line of work by my assistant, Mr. L. L. Hardt.

the constant presence of a small amount of ammonia in the pure gastric juice (appetite secretion) in the dog. Reisner (29) concludes that the ammonia in gastric juice comes from the saliva.

What is the origin and significance of the gastric juice ammonia? It is known that saliva contains traces of ammonia. We find that the mixed saliva of man (A. J. C.) contains from 0.5 to 1.5 mgr. ammonia per 100 cc. Salaskin found 2.5 mgr. NH_3 per 100 cc. in the saliva of the dog. But in dogs with Pawlow's stomach pouch, and in my gastric

fistula case, Mr. V., no saliva can enter the stomach or the part of the stomach yielding the juice. In the present work the saliva therefore is not a factor.

The ammonia of the duodenal content may be a factor, as Boldyreff has shown the frequency with which intestinal content enters the stomach. This factor is excluded in dogs with the Pawlow stomach pouch. In my human fistula case this factor is readily controlled by making the ammonia determinations only on those samples of gastric juice that are absolutely free from admixture with bile, pancreatic juice, and succus entericus.

Rosemann points out that the gastric juice ammonia cannot be a simple filtrate from the blood since normal blood contains only about 0.5 mgr. of ammonia per 100 cc.

The above considerations seem to limit the origin of the gastric juice ammonia to the following factors:

1. It may be an active excretion from the blood, in which case one would expect an increase in the gastric juice ammonia on increasing the blood ammonia. In normal individuals there appears to be a decrease in the elimination of ammonia by the kidneys during gastric secretion (Gammeltoft (28).

2. The ammonia may be formed in the secretion process itself.

3. The action of the hydrochloric acid on the proteins of the gastric juice (conversion of alkaline or neutral proteins to acid proteins).

4. The action of the hydrochloric acid on cells of the mucosa (splitting off of ammonia by the cells as a protective measure against the action of strong acids).

5. In the case of gastric ulcers of infectious origin ammonia may actually be produced continually by bacterial action in the active focus of the ulcer.

6. In the case of traumatic or non-infectious ulcers it seems highly probable that the action of the hydrochloric acid on the raw surfaces of the gastric mucosa will result in the formation of ammonium chloride.

In 1898 Nencki, Pawlow and Zaleski (20) reported studies on the ammonia concentration in the gastric mucosa and its relation to the secretion of gastric juice. They found that, per unit of mass, there is more ammonia in the gastric mucosa than in any other tissue of the body. 100 gr. gastric mucosa at rest contained 20 mgr. NH_3. 100 gr. gastric mucosa after 2 hours secretion (sham feeding) contained 42 mgr. NH_3.

These findings were essentially confirmed by Salaskin the same year. They seem to indicate some relation of the ammonia formation to the

secretion process itself, unless the higher ammonia content of the secreting mucosa represent ammonium chloride in the process of absorption from the gastric juice. The other possible factors referred to above are capable of experimenta tests and work is in progress in that line.

3. *The amino-acids of the gastric juice.* The formol titration was made on eight lots of appetite gastric juice of Mr. V., and on three lots of appetite gastric juice of Mr. E. When deductions were made for the ammonia nitrogen of the juice, the formol titrable nitrogen of Mr. V.'s gastric juice varied from 3 to 9 mgr. nitrogen per 100 cc. Mr. E.'s appetite juice gave 7 mgr. per 100 cc. Four lots of dog's appetite gastric juice (Pawlow pouch) gave only 1 to 2 mgr. of amino-acid nitrogen. It thus appears that normal human gastric juice contains slightly more amino acid than ammonia nitrogen; but the greater part of the gastric juice nitrogen is associated with the more complex proteins.

Zunz (19) working with gastric contents after test meals reports that the amino acid nitrogen usually exceeds the ammonia nitrogen, and that both substances are increased in cases of gastric cancer. In three normal persons the maximum amino acid nitrogen was 10 mgr. per 100 cc. of gastric content, while in several gastric cancer cases it reached 15–20 mgr. per 100 cc. of content. But these figures cannot be directly compared with ours on pure gastric juice, because of the uncertain factors associated with the gastric contents following a test meal.

4. *The auto-digestion of the gastric juice.* When fresh gastric juice is incubated at 38° C. the following changes take place in the proteins and the gastric mucin.

(1) All the ropy mucin and mucin flocculi are dissolved.

(2) The pink color of the biuret reaction is increased. In fact, fresh human gastric juice gives practically a violet biuret reaction, and this color is intensified and changed towards pink by the auto-digestion.

(3) The characteristic protein precipitation at the point of neutralization is decreased.

(4) The quantity of proteins precipitated by nitric acid and by heat is reduced.

It is thus clear that the proteins of pure gastric juice undergoes pepsin-hydrochloric acid digestion in the stomach itself. But some of the gastric juice proteins are not hydrolyzed, at least not down to the peptone stage. It has been pointed out in a previous report (1) that this auto-digestion of the gastric juice itself is probably a factor in the continuous secretion of gastric juice in the way of yielding gastric secretagogues.

TABLE IV

Anaphylactic reactions of guinea pigs, using human gastric juice and human serum as sensitizing and toxic agents. Pigs 7, 8 and 9 were sensitized with human gastric juice that had been incubated at 38°C. for 10 days

G. PIG	SENSITIZING DOSE	INTERVAL	TOXIC DOSE	ANAPHYLACTIC SYMPTOMS
1	0.1 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
2	0.2 cc. fresh g. juice	15 days	4 cc. fresh g. juice	Doubtful
3	0.5 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
4	1.0 cc. fresh g. juice	15 days	4 cc. fresh g. juice	Doubtful
5	1.5 cc. fresh g. juice	15 days	2 cc. fresh g. juice	Doubtful
6	2.0 cc. fresh g. juice	15 days	2 cc. fresh g. juice	None
7	2.0 cc. digested g. juice	20 days	1 cc. human serum	Severe; death in 1¼ hrs.
8	1.0 cc. digested g. juice	20 days	3 cc. fresh g. juice	None
9	0.2 cc. digested g. juice	20 days	3 cc. fresh g. juice	None
10	1.0 cc. fresh g. juice	23 days	0.5 fresh human serum	Severe; death in 1 hr.
11	2.0 cc. fresh g. juice	23 days	5.0 fresh g. juice	Doubtful
12	5.0 cc. fresh g. juice	23 days	5.0 fresh g. juice	Slight
13	0.5 cc. fresh g. juice	27 days	5.0 fresh g. juice	Doubtful
14	1.0 cc. fresh g. juice	27 days	0.5 cc. human serum	Severe; recovered
15	5.0 cc. fresh g. juice	27 days	5.0 fresh g. juice	None
16	0.5 cc. fresh g. juice	30 days	0.5 cc. human serum	Severe; recovered
17	1.0 cc. fresh g. juice	30 days	5.0 fresh g. juice	Doubtful
18	2.0 cc. fresh g. juice	30 days	5.0 fresh g. juice	None
19	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
20	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
21	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
22	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
23	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
24	10.0 cc. fresh g. juice	18 days	1.0 cc. human serum	Severe
25	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
26	5.0 cc. human serum	18 days	10.0 fresh g. juice	Doubtful
27	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
28	5.0 cc. human serum	18 days	10.0 fresh g. juice	None
29	0.05 cc. human serum	27 days	1 cc. human serum	Severe; death in 1½ hrs.
30	0.05 cc. human serum	27 days	10.0 fresh g. juice	No symptoms
31	0.10 cc. human serum	27 days	2.0 cc. human serum	Severe; recovered
32	0.10 cc. human serum	27 days	10.0 fresh g. juice	None
33	0.15 cc. human serum	27 days	2.0 cc. human serum	Severe; recovered
34	0.15 cc. human serum	27 days	10.0 fresh g. juice	Doubtful
35	0.25 cc. human serum	27 days	1.0 cc. human serum	Severe; death 1 hr.
36	0.25 cc. human serum	27 days	10.0 fresh g. juice	None
37	0.5 cc. human serum	27 days	1.0 cc. human serum	Severe; death 1 hr.
38	0.5 cc. human serum	27 days	10.0 fresh g. juice	None

5. *The nature of the gastric juice proteins and their relation to the serum proteins as shown by the anaphylactic reaction.*

This series of experiments on guinea pigs with human gastric juice as a sensitizing and toxic dose for anaphylaxis were made as a preliminary step for the study of pathological gastric juice. The injections were made intraperitoneally with a blunt needle. The gastric juice was rendered slightly alkaline by titration with $\frac{N}{10}$ NaOH. It is well known that at the neutral point of the gastric juice some of the proteins are precipitated, and that these go into solution on rendering the juice slightly alkaline. The human serum was obtained, not from Mr. V. who yielded the gastric juice, but from Mr. Rogers, who assisted in this part of the work.

The results are summarized in Table IV. These data seem to show that: (a) *Normal human gastric juice contains a sensitizing substance for human serum, but that it is practically devoid of toxic substances both in relation to the proteins of the gastric juice and to serum proteins.* This is rather an unexpected situation, in view of the fact that the gastric juice contains complex proteins in considerable amounts. (b) The gastric juice contains some practically unchanged serum proteins capable of acting as sensitizer, but incapable or in too small concentration to act toxically. This sensitizing substance is not diminished or destroyed by auto-digestion of the gastric juice at 38° C. for ten days (Table IV, pigs 7, 8, 9).

It is of interest in this connection to recall that according to Gay and Adler (22) the euglobulin (of horse serum) is sensitizing but not toxic. And Wells (23) has shown that enzyme (trypsin) hydrolysis of proteins destroys the toxic properties of the proteins very must faster than the sensitizing properties, the latter persisting even after three years continued tryptic digestion.

V. *The acidity of normal gastric juice.* The acidity was determined by titration with $\frac{N}{10}$ NaOH, and using dimethyl-amino-azo-benzene and phenolthalein as indicators for the free and the total acidity respectively. During the three years that Mr. V. has been under observation hundreds of determinations have been made of the acidity of the contents of the "empty" stomach, of the hunger juice or continuous secretion, and of pure appetite juice. The reader will recall that the contents of the "empty" stomach is taken one hour after washing out the stomach with 200 cc. of water. All the cases where the gastric juice or gastric content was contaminated with bile (intestinal content) are excluded from the summaries given in Table V.

The second gastric fistula case, Mr. E., is a man 26 years of age, healthy and vigorous. Nearly a year ago his oesophagus was corroded with a solution of lye, and this led to a nearly complete cicatricial stenosis. The gastrostomy was made in October, 1914. I am under obligations to Dr. Bayard Holmes for the privilege of working with Mr. E. daily for two weeks. My observations were made in February, 1915. At that time the oesophagus had been dilated sufficiently to permit swallowing of any well masticated food and the gastrostomy opening was used only in the dilation processes. In this case saliva is therefore not excluded from the contents of the empty stomach, and possibly not from the continuous or hunger secretion, although Mr. E. was instructed and urged not to swallow any saliva during these experiments. The appetite juice was obtained by Mr. E.

TABLE V

The acidity of normal human gastric juice

PERSON	MATERIAL	NO. OF OBSERVATIONS	ACIDITY					
			Free			Total		
			Low	High	Aver.	Low	High	Aver.
Mr. V.	Cont. empty stomach.	235	0.10	0.35	0.18	0.15	0.40	0.23
	Hunger juice.....	180	0.15	0.35	0.25	0.20	0.45	0.34
	Appetite juice.....	285	0.35	0.44	0.40	0.40	0.53	0.48
Mr. E.	Cont. empty stomach.	10	0.09	0.36	0.20	0.18	0.41	0.25
	Hunger juice.....	8	0.20	0.32	0.25	0.27	0.38	0.33
	Appetite juice.....	15	0.30	0.36	0.34	0.36	0.47	0.44

chewing palatable food, and spitting out the chewed food, care being taken not to swallow saliva or particles of food.

These results on my two gastric fistula cases are in agreement with the work of Pawlow and his pupils on dogs, and the work of previous observers on pure gastric juice of normal persons. The latter data have recently been brought together and discussed by Boldyreff (24).

Normal human gastric juice (appetite secretion) when secreted above a certain minimum rate shows a practically constant total acidity of nearly 0.5 per cent HCl, or the same as the gastric juice of normal dogs. The gastric juice (appetite as well as hunger juice) secreted by the normal stomach at a low rate shows lower than normal acidity and total chlorides. The view of Pawlow based on experiments on dogs that gastric juice is secreted at uniform and constant acidity is true for man only

in regard to the appetite, digestion, and hunger juice secreted at fairly high rate. We must take cognizance of the equally important fact that the normal gastric mucosa is capable of secreting a juice of submaximal acidity.

Last year Rehfus and Hawk (25) reported a series of interesting observations on the acidity of the gastric content at varying periods after drinking water, and ingesting an Ewald test meal, which they offer as "direct evidence of the secretion of gastric juice of a constant acidity in the human stomach," and as "the first direct demonstration in the human subject in favor of Pawlow's theory of the secretion of a juice of a constant acid concentration." Rehfus and Hawk are dealing with the gastric content, not with pure gastric juice. The fact that the gastric content soon reaches and then maintains for some time a fairly constant acidity indicates the equilibrium between the acid secretion and the factors of neutralization, as shown by Boldyreff, but it does not show that the juice is poured into the stomach at a certain constant acidity. Analysis of the Ewald meal or the continuous secretion do not permit us to draw any conclusion in regard to the acidity of the actual gastric juice. If the authors mean by the above conclusions that the gastric juice of normal persons, that is the pure appetite juice, or the juice secreted during the first two or three hours of digestion exhibit a fairly constant acidity of nearly 0.5 per cent HCl, they are undoubtedly correct; but this conclusion is not new, as shown by Boldyreff's review of the earlier literature, and it cannot be based on the observations they report.

The reader will note that *normal human gastric juice is equal in total acidity to the maximum acidity reported by clinical observers for so-called hyperacidity in man.* So far as I am acquainted with the literature, *there is no evidence that the gastric glands under any pathological conditions are able to or do secrete a juice of higher than normal acidity.* Moreover, the presence in the stomach of gastric juice of full acid strength leads by itself and immediately to no untoward symptoms.

The contents of the "empty" stomach, and the continuous or hunger secretion (when the secretion rate is low) have uniformly a lower acidity than the appetite juice. The total acidity of contents of the "empty" stomach is about 0.2 per cent. The reader will note that this figure is frequently given, especially by clinicians, as the acidity of pure gastric juice of normal persons. The acidity of the continuous or hunger secretion is higher, and the greater the secretion rate the higher the acidity until it may equal that of the appetite juice. In no instance

does the acidity of the continuous secretion exceed that of the appetite juice.

What is the cause of the low acidity of the continuous secretion and contents of the empty stomach? The following factors must be taken into account:

1. The actual acidity of the juice as secreted may increase with the secretion rate, until the maximum acidity is reached with the high average rate of secretion, a condition similar to that obtaining in the case of the salivary glands where the concentration of the salts and the organic materials increase with the rate of a salivary secretion. If this is a factor the gastric juice secreted at a low rate should show a lower osmotic concentration and total chlorides than the juice secreted at high rate. The figures reported by Umber (12) for man and by Rosemann (2) for the dog appear to support this view, the former investigator showing particularly that the osmotic concentration (Δ) of the gastric juice increases with the rate of secretion. The cryoscopic data may, however, be misleading, as the salts produced by the neutralization of the HCl may not dissociate as freely as the acid.

2. The slower rate of secretion may give chance for the HCl to be partly neutralized by the alkaline mucus secreted by the mucin cells of the gastric mucosa. This is the factor emphasized by Pawlow. In fact, Pawlow takes the position that in the normal animal gastric juice has practically a constant acidity, irrespective of the secretion rate, but the actual acidity of the juice in the cavity of the stomach is purely a matter of rate of neutralization. If this is the sole factor, the total chlorides of the gastric juice ought to show a greater constancy than the acidity. That the hydrochloric acid of the gastric juice is in part neutralized by the gastric mucus is obvious. But according to Boldyreff the alkalinity of gastric mucus is only 0.05–0.10 per cent Na_2CO_3. That is to say, it would require 50–100 cc. gastric mucus to reduce 100 cc. gastric juice from the normal acidity of 0.45 per cent down to 0.25 per cent. The importance of this factor has therefore been overestimated by Pawlow.

3. When the gastric juice is collected from a Pawlow accessory stomach, or from an individual with complete closure of the oesophagus, as is the case with Mr. V., the saliva cannot be a factor in lowering the gastric juice acidity by neutralization and dilution. When all or most of the saliva is swallowed the acidity of the gastric juice is necessarily reduced in proportion to the relative rate of salivary and gastric secretion. This is effected by dilution rather than by neutralization,

as the titration alkalinity of saliva is low (0.08 per cent Na_2CO_3, Neumeister, cited by Boldyreff).

4. According to Boldyreff the most important factor in lowering the acidity of gastric juice from that actually secreted by the gland (0.5 per cent) to that usually found in the cavity of the stomach (0.25 per cent) is the entrance of intestinal contents (pancreatic juice, bile, and succus entericus) into the stomach. Boldyreff has shown that this occurs in dogs, and probably occurs in man, when the acid in the stomach mounts much above 0.25 per cent. I am satisfied from my observations on Mr. V. that Boldyreff's view is essentially correct. When 15–25 cc. appetite gastric juice of full normal acidity is permitted to remain in the empty stomach, even a few minutes, the pyloric sphincter dilates, and the contents of the upper end of the duodenum is in some way forced into the stomach. The quantity of intestinal content entering the stomach at any one time varies from a trace up to 10 cc., and the acidity of the gastric juice is correspondingly reduced by dilution and neutralization. The presence of duodenal content in the empty stomach is therefore a normal occurrence, but the mechanism involved in this intestinal regurgitation has not been closely worked out. We know from the work of Cannon and others that an acid reaction on the stomach side of the pylorus tends to dilate the pyloric sphincter. But it is difficult to see how a patent pylorus can cause the pancreatic juice and bile to enter the stomach without an actual antiperistalsis in the duodenum.

It is clear that it is this "mechanism for self regulation of the acidity of the stomach content" (Boldyreff) which breaks down in cases of so-called "hyperacidity" in man. In cases of "hypersecretion" the quantity of juice secreted is greater than normal, and the secretion may persist in the absence of all normal stimuli, but the neutralizing factors suffice to reduce the acidity of the juice approximately to that found in the normal stomach. It is purely a balance of secretion rate and of neutralization capacity. Impairment of the neutralization factors or very excessive secretion rate of gastric juice would tend to render the acidity of the gastric content equal to that of pure gastric juice; in other words, produce clinical "hyperacidity."

VI. *The total chlorides of the gastric juice.* The total chlorides of the gastric juice were determined by the method of Volhard on 10 different lots of appetite juice and 11 different lots of the hunger juice or continuous secretion. The results are summarized in Table VI. The secretion rate and the acidity of the various lots of juice are also

recorded. It will be seen that the total chlorides of the appetite juice are very constant, the minimum being 0.49 per cent and the maximum 0.56 per cent chlorine. The continuous secretion or hunger juice is more variable in chloride content, and this variation appears to be directly dependent on the secretion rate and on the acidity. In general the lower the secretion rate the lower the acidity and the lower

TABLE VI

The total chlorides of normal human gastric juice

NO. OF EXP.	GASTRIC JUICE	SECRETION RATE PER HR.	ACIDITY %		TOTAL CL. %
			Free	Total	
		cc.			
1.....	Hunger juice	6	0.20	0.32	0.40
	Appetite juice	560	0.42	0.48	0.56
2.....	Hunger juice	4	0.18	0.25	0.25
	Appetite juice	308	0.41	0.48	0.53
3.....	Hunger juice	8	0.30	0.40	0.38
	Appetite juice	500	0.41	0.49	0.54
4.....	Hunger juice	12	0.30	0.41	0.44
	Appetite juice	398	0.42	0.49	0.55
5.....	Hunger juice I	2	0.20	0.32	0.28
	Appetite juice	154	0.42	0.48	0.56
	Hunger juice II	10	0.29	0.38	0.36
6.....	Hunger juice	2	0.20	0.33	0.34
	Appetite juice	200	0.41	0.46	0.53
7.....	Hunger juice	6	0.30	0.41	0.43
	Appetite juice	280	0.45	0.52	0.56
8.....	Hunger juice.....	3	0.18	0.28	0.27
	Appetite juice	150	0.41	0.47	0.53
9.....	Hunger juice	2	0.14	0.29	0.24
	Appetite juice	210	0.43	0.50	0.56
10.....	Hunger juice	3	0.26	0.36	0.36
	Appetite juice.....	100	0.32	0.40	0.49
11.....	Hunger juice	36	0.34	0.40	0.53
	Appetite juice (mixed with bile)	228	0.32	0.37	0.56

the total chlorides. This is in agreement with the findings of Foster and Lambert (26) on dogs.

These facts seem to point to the conclusion that the low acidity of the gastric juice secreted at a slow rate is not due entirely to neutralization by alkaline mucus. We have apparently a secretion of gastric juice of an acidity actually lower than that of the rapidly secreted

appetite juice. The dependence of the actual secreted acidity on the secretion rate is not a very close one, however, as we may have very marked fluctuation in rate without any change in chlorides. But below a certain secretion rate (25–30 cc. per hour from the entire stomach of the adult) an actual hypoacid juice is secreted.

The above figures for total chlorides in normal human gastric juice agree closely with the findings of previous observers on the gastric juice of dog and of man. Rosemann gives 0.54–0.64 per cent Cl for the appetite gastric juice of the dog. The figures given by Sommerfeld for human appetite juice varies from 0.53 to 0.59 per cent Cl. Umber working on an old man (59 years) with partial esophageal stenosis (malignant) reports total chlorides of the gastric juice as varying from 0.27 to 0.60 per cent Cl. The reader will note that the lowest figure reported by Umber is practically identical with the lowest total chlorides found by me in the very slowly secreted hunger juice. It is probable that the low chloride gastric juice samples of Umber were secreted at a slow rate.

VII. *The concentration of pepsin.* Most of the pepsin determinations were made by the method of Mett; a few tests were also made with the ricin, and the U. S. Pharmacopœia methods. The tubes (2–2½ mm. diameter) were sealed at both ends with heat and the egg albumin coagulated by boiling for 10 minutes, and the tube left in the water until cooled, and then set aside for two days before using.

The digestion mixture was made up as follows: 1 cc. gastric juice + 15 cc. $\frac{N}{10}$ HCl, + two Mett's tubes (1½ cm. length) at 37° C. for 24 hours. The $\frac{N}{10}$ HCl was used because it approximates the free acidity of appetite gastric juice, although we are aware of the fact that this degree of acidity is somewhat higher than the optimum for artificially prepared pepsin (cf. Cobb (27), Sörensen (30), Michaelis and Davidsohn (31)). We propose to determine the optimum acidity for the pepsin in normal gastric juice in a later work. It would seem singular if normal gastric juice is secreted with an acidity too great for optimum peptic digestion. It may be so, however, for under normal conditions the actual peptic digestion must be carried out under less than normal acidity of the gastric juice, because of dilution with saliva and the water of the food, and the fixation of the acid by the protein of the food.

Our results with the Mett's test on the gastric juice of Mr. V. may be given in the following summary:

Appetite gastric juice.
Number of tests (Lots of G. J.): 45.
Digestion in mm.: High, 8½; low, 6; *average*, 7.
Hunger juice (continuous secretion):
Number of tests: 35.
Digestion in mm.: High, 8½; low, 5; *average*, 6.
Contents of the "empty" stomach:
Number of tests: 35
Digestion in mm.: High, 4; low, 3; *average*, 3½.

The results are stated in the length of albumin column actually digested, because according to Cobb the law of Schütz does not hold for pepsin in concentrations that digest more than 4–5 mm. in 24 hours.

The variations in pepsin concentrations in these three groups of

TABLE VII

The relation of total acidity and pepsin concentration (Mett's method) in normal human gastric juice (Mr. V).

SERIES	CONTENTS "EMPTY" STOMACH		HUNGER G. JUICE		APPETITE G. JUICE	
	Acidity	Pepsin	Acidity	Pepsin	Acidity	Pepsin
	per cent	*mm.*	*per cent*	*mm.*	*per cent*	*mm.*
18	0.18	4	0.40	6	0.48	7
28	0.10	3	0.25	6	0.48	7½
35	0.18	3½	0.41	6½	0.47	7
38	0.15	4	0.38	7	0.48	7½
45	0.15	4	0.40	6½	0.49	7½
95	0.22	4	0.28	6½	0.41	7
100	0.10	3	0.35	6	0.44	6½

gastric juice, and the absence of a close relation of pepsin concentration and acidity is further illustrated by a few typical series of tests given in greater detail in Table VII.

The appetite gastric juice of Mr. E., our second gastric fistula case, when tested as above in 14 experiments showed a pepsin concentration of 5–7 mm. with an average of 6 mm., a slightly lower value than the gastric juice of Mr. V.

We have tested as above the gastric juice (appetite, as well as digestion secretion) of 25 dogs with Pawlow fistula. The dogs, normal and in good condition, were being used in other lines of work. We were surprised to learn that the dog's gastric juice showed uniformly a lower pepsin concentration than the gastric juice of man. The figures ob-

tained on dog juice was: highest, 5 mm.; lowest, 2 mm., with an average of $3\frac{1}{2}$ mm. The reader will note that this is only about half the quantity of digestion obtained in the human juice.

When the Mett's tubes are placed in 16 cc. of undiluted human gastric juice (appetite secretion) the digestion in 24 hours at 37° C. varies from 12–16 mm., or only twice the quantity digested in the dilution of 1 cc. juice to 15 cc. $\frac{N}{10}$ HCl. This seems to indicate that *in normal gastric juice the pepsin is present in excess of the needs or at least far in excess of that needed in economic digestion.*

The U. S. Pharmacopœia defines "100 per cent Pepsin as a preparation capable of digesting three thousand times its own weight of finely divided egg white (coagulated) in three hours." The Pharmacopœia test is carried out as follows: 10 grams of boiled white of egg is macerated through a No. 40 filter and placed in 40 cc. 0.3 per cent HCl, $3\frac{1}{3}$ mgr. dried pepsin added, and the mixture incubated at 52° C. for three hours, with occasional stirring When thus carried out there is only a very small residue of undissolved egg white at the end of three hours, but the procedure to measure the amount of this residue does not yield very accurate results.

This test was applied to six different lots of appetite gastric juice of Mr. V. Under above conditions, 1–$1\frac{1}{2}$ cc. appetite gastric juice digest 10 gr. of coagulated and finely divided egg white in three hours practically as completely as is done by $3\frac{1}{3}$ mgr. "100 per cent pepsin." As defined by the U. S. Pharmacopœia, 1 cc. of human gastric juice must therefore contain $3\frac{1}{2}$ mgr. pepsin, or 100 cc. of the juice, 35 mgr. pepsin. We have seen that the appetite gastric juice of man contains about 400 mgr. organic material per 100 cc. That is, according to the Pharmacopœia definition, only about 10 per cent of the organic matter in the human gastric juice is pepsin. Of course, we are aware of the fact that the Pharmacopœia definition and test is only one of convenience for standardizing commercial preparations of pepsin, and having no direct relation to pepsin concentration in normal gastric juice.

It has been shown in a previous report that an adult normal person if hungry secretes 600–700 cc. gastric juice on an average palatable dinner, or about 1500 cc. gastric juice total in 24 hours. That is to say, there is a secretion of 240–250 mgr. pepsin per dinner, capable under proper conditions of digesting from 630 gr. to 750 gr. of protein (coagulated and finely divided egg albumen) in three hours; and the total pepsin secretion in 24 hours is 525 mgr., capable of digesting $1\frac{1}{2}$ kilo proteins (coagulated egg white) in three hours.

The practically complete digestion of 10 gr. boiled and finely divided egg white by 1 cc. human gastric juice in three hours is a fact. The secretion of 600–700 cc. gastric juice on an avergage dinner by the adult human stomach is an estimate, but not far from the actual fact. It is therefore clear that the normal human stomach secretes pepsin far in excess of the *actual needs* of gastric digestion, or, more precisely, far in excess of what can be used advantageously under ordinary conditions of gastric digestion When the boiled egg white is broken up in larger pieces, such as occurs in ordinary rapid mastication, 1 cc. of gastric juice requires 6–10 hours for complete digestion.

This great excess of pepsin in normal gastric juice probably explains the clinical findings of great reduction in pepsin content without any evidence of impaired gastric digestion, provided sufficient acid is present. It probably also explains, in part at least, the practical uselessness of commercial pepsin as a therapeutic measure in gastric disorders.

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THE CONTENT OF SUGAR IN THE BLOOD OF CATS UNDER THE INFLUENCE OF COCAINE

EDWARD WALDO EMERSON SCHEAR

From the Department of Physiology of Columbia University

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It has become apparent that the chemical composition of the blood is very rapidly changed by the conditions to which animals are subjected both preliminary to and during the operation of drawing samples of blood. Especially is this true with reference to the carbohydrates.

Thus Pavy (1) shows not only that it is necessary that the animals be kept free from excitement while the sample is being drawn, but also that when the animal is killed and all the blood is used, ". . . it is necessary to proceed with the utmost expedition in order that no change may take place in the contents of the circulation from the *post mortem* production of sugar in the liver." These results have been substantiated by many investigators since the publication of Pavy's work and are treated in considerable detail in a recent paper by Scott (2), in which the results of extensive researches are given, with numerous references to the literature both on glycaemia and glycosuria. Still more recently Shaffer (3) shows that the sugar content of the blood of the dog when "free from excitement or pain is surprisingly low and constant." Scott also discusses the effects of chloroform and ether, and records a few results from the use of cocaine.

It is the purpose of this paper to put the effects of cocaine to a more thorough analysis by means of a larger series of experiments. The chemical technique used in the experiments here recorded is the same as that described by Scott in the paper above referred to, and need not be repeated. The work was performed under the immediate direction of Dr. Scott, and it is a pleasure to acknowledge my indebtedness to him.

DESCRIPTION OF EXPERIMENTS

In these experiments rather large doses of cocaine hydrochloride dissolved in M/8 sodium chloride solution were given. It is obvious

that if results to which any reliability can be attached are to be expected, certain standard conditions must be determined upon which the effect of the drug itself can be superimposed. The conditions adopted in this work are the same as those reported by Scott in the paper above referred to, pages 278 to 293. The chief essentials are that the animals were kept in captivity for from ten to twenty days under excellent conditions and were fed exclusively on meat, the natural diet of the cat.

It is well known that certain other processes of metabolism are affected by cocaine injections; therefore it was necessary to proceed with extreme caution in the carrying out of the experiments, as well as in their interpretation. For example, Araki (4) found that lactic acid was eliminated in the urine of frogs and rabbits after cocaine injections; also one rabbit out of four secreted sugar with the urine. Underhill and Black (5) report that while small doses of cocaine, 10 mgm. per kilo of body weight, cause no appreciable influence upon the course of nitrogenous metabolism or the utilization of protein and fat, larger doses, 15 to 20 mgm. impair, first, fat utilization and, subsequently, that of protein, while a marked decline in body weight is manifest. It has also been shown by Scott and others that the character of the diet upon which animals are fed will affect the sugar concentration. Besides this it is quite evident from the work of a number of investigators that the glycosuria which has been found in many cases, as well as the hyperglycaemia which is known to exist even before any sugar is excreted, is frequently due to the emotional condition of the animal entirely apart from the diet or the anesthetic.

For these and other reasons only such animals as conformed to the standard conditions were used in these experiments. Throughout the work great care was taken to avoid everything that would in any way tend to excite the animals before drawing the blood for analysis. It was of course impossible to do this with absolute success in every case, inasmuch as some of the cats which it was found necessary to use were unaccustomed to being handled, even though not particularly wild. Others were somewhat wild. When, however, anything occurred that might indicate other causes than that of the drug for hyperglycaemia strict records of such conditions were made. The animals were killed by sudden decapitation, and the blood was obtained from the severed vessels of the neck.

In table 1 are given the results from five animals which were almost entirely quiet throughout the handling preliminary to the injection

and during the operation itself. It will be noticed that the amounts of sugar in the blood here recorded are very close together, the highest variation from the mean being only 15 per cent. In fact the variation is but little greater than that found by Scott in normal cats, or than is to be expected from unavoidable variations in the internal relationships with their necessary readjustments, as is pointed out by Cannon (6).

TABLE 1

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	VAR. FROM MEAN	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*		
1	F	18	2.80	0.070	6	62.83	0.0736	+11	Fairly quiet
2	F	18	3.30	0.070	6	79.85	0.0720	+ 9	Quiet
3	F	12	3.00	0.050	10	77.38	0.0679	+ 2	Ideal
4	F	14	2.20	0.050	10	50.11	0.0619	− 7	Practically quiet
5	F	17	1.90	0.050	8	49.90	0.0546	−15	Slightly excited before, but quiet during injection
Mean							0.0664		

The results from the four excited animals reported in table 2 would scarcely be expected to vary greatly from those of table 1, since the excitement, while evident, was not at all excessive. The mean is somewhat higher than the mean of table 1, which result is in accord with the results obtained by various investigators already referred to.

TABLE 2

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	VAR. FROM MEAN	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*		
6	F	18	2.6	0.045	11	81.45	0.0815	+ 9	Excited from first Cried during injection
7	F	12	2.3	0.050	5	58.63	0.0643	−14	Cried during injection
8	M	14	2.8	0.050	5	80.00	0.0832	+11	Restive
9	M	17	2.15	0.050	6	64.65	0.0695	− 7	Restless; slightly excited
Mean							0.0746		

This conclusion is strengthened by the interesting examples of the two individuals recorded in table 3. These animals showed exceptional reaction to the drug. In the first there was no particular excitement manifest before the injection, but as soon as the operation was over marked excitement was noticed.

Protocol of Experiment 10

Time of injection 10.05. Quantity, 27 mgm. per kilo of body weight.
10.08. Cries, due to drug; had not been excited.
10.09. Evidence of nausea; deep respiration; vomiting.
10.10. Pupil reflex slow.
10.12. Hind legs weak; respiration rapid; cries; salivation.
10.15. Spasms; after about 10 seconds the first spasm diminished slightly, and the animal was killed just as the second spasm set in; blood seemed cyanotic.

TABLE 3

NO. OF EXP.	SEX	DAYS ON DIET	BODY WT.	AMT. COC. INJ.	TIME FROM INJ. TO DEATH	AMT. BLOOD DRAWN	% SUG. IN BLOOD	REMARKS
			k.	*gm.*	*min.*	*gm.*	*gm.*	
10	F	11	2.6	0.070	10	54.52	0.1393	Quiet at start; drug showed marked influence; nausea and spasms.
11	F	11	2.5	0.035	27	82.92	0.2215	Cat fought during injection

In the other experiment, No. 11, the cat was difficult to handle and fought from the first. The injection was not entirely successful, only about one-half of the syringeful of cocaine being introduced. Since the cat was rather large the injection was thought hardly sufficient to yield adequate results, hence the animal was rejected and returned to the cage. In a short time, however, the characteristic symptoms were manifest: intense salivation, dilated pupils, etc. The cat was killed twenty-seven minutes after the injection. It will be noticed that the result in No. 10 was higher than in any other except No. 11, yet the quantity of cocaine used in the former was approximately the same as that in other cases; in the latter case the quantity used was only about half as great, yet the carbohydrate recovered is extremely high. The reason why the result in No. 11 is higher than in No. 10 is possibly twofold. In the first place the excitement manifested by the cat was very much greater, and, secondly, the time between the injection and the killing was longer. This, however, merely substantiates the results of a number of investigators previously reported.

CONCLUSIONS

1. If we compare the amount of sugar in the blood of cocainized cats in table 1, mean 0.0664, with Scott's standard of 0.069 for normal animals, it is seen that the mean of the series here presented is slightly lower than the standard. This might be taken to indicate that the direct influence of the cocaine tends to diminish the concentration of the carbohydrate in the blood, and is thus in harmony with the short series reported by Scott. This difference, however, is so slight that for most purposes it may be neglected.

2. While an increased concentration of sugar was noted in the blood of several cocainized animals it seems obvious that this was due to excitement. It seems a matter of indifference whether this excitement is due to the action of the drug or to other causes. In this connection it may be noted that while the cocaine may truly cause hyperglycaemia it does so only in an indirect manner.

3. While cocaine may be used for local anesthesia, in drawing blood for sugar analyses its use involves an element of doubt that would better be obviated if possible.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXVI. The Relation between the Digestion Contractions of the Filled, and the Hunger Contractions of the "Empty" Stomach

F. T. ROGERS and L. L. J. HARDT

From the Hull Physiological Laboratory of the University of Chicago

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Boldyreff in 1905 (1) first reported that there occurred contractions of the empty stomach of the dog during starvation. Cannon and Washburne (2) reported that the same occurred in man. Carlson (3) by more delicate methods, analyzed the various types of motor activities of the empty stomach during prolonged hunger. His work showed that the empty stomach exhibits three types of activity.

1. Rhythmical contractions occurring about three times per minute designated the "twenty second rhythm."

2. Very vigorous contractions occurring periodically. The duration of each of these contractions is about thirty seconds.

3. Tonus changes of the stomach musculature.

In his first paper Carlson states that probably the twenty second rhythm is the peristaltic activity of the antrum pylori. The thirty second rhythm or hunger contractions were thought to be contractions of the fundus. His later work on dogs with Pawlow pouches showed that the twenty second rhythm is not confined to the pylorus, but is also exhibited by the fundus (4). The general trend of all of Carlson's experiments is toward the conclusion that the first period of hunger contractions apparent after a meal is an exaggeration of the motor activities of the stomach occurring during gastric digestion.

In experiments on the rabbit (5) undertaken at Dr. Carlson's suggestion it was found difficult to differentiate the hunger contractions of the stomach from the digestion peristalsis. No other conclusion seemed possible than that the hunger contractions were augmented peristaltic contractions.

In conference with Dr. Carlson the following experiments were outlined to determine whether or not the same conclusion applied to man and dog:

We used the two standard methods of investigating the movements of the stomach: the rubber balloon and the X-ray methods. To study the genesis of the hunger contractions the rubber balloon, which was connected by small size rubber tubing with the recording manometer, was swallowed shortly after a meal and continuous graphic records of the intragrastric pressure variations were made until after the onset of a typical hunger period.

Soon after eating an average meal the subject of the experiment swallowed the balloon and put himself into a comfortable position either sitting in a chair or lying on a cot. The results were similar, irrespective of the position; the best results were obtained while the subject was asleep for then inhibiting psychic influences were removed. During a long continued series of tracings we tried to keep the balloon in the upper part of the stomach by pulling it up at intervals and swallowing it again. We realize it is possible for it to be pushed to the pyloric end of the stomach, but by the X-ray observation in the dog we found that the balloon introduced after feeding remained in the cardiac end of the stomach for a considerable time.

In order to actually see the hunger movements we coated a balloon with a bismuth paste and observed its movements in the stomach with the X-ray.

The only practicable way we found of preparing these balloons consisted in painting the outer wall of one balloon with a bismuth paste prepared by mixing bismuth subnitrate with vaseline. This balloon was then inclosed within another of the same size; thus the two balloons were separated by a thin wall of bismuth paste. These balloons had to be prepared anew for each experiment, as the peristaltic waves tend to sweep all the bismuth to the lower end of the balloon and the vaseline soon causes the rubber to lose its elasticity. We were able to make graphic tracings with simultaneous direct fluoroscopic observation.

Our records show that the fundus is quiescent immediately after eating a meal. The pressure upon the balloon is maintained at a steady level. If a light meal is taken the tonus variations may be demonstrated immediately after eating. At first they are so slight as to seem insignificant but they increase in vigor and are usually visible thirty minutes after the meal. In one experiment an unusually large meal was eaten and the tonus waves were distinctly in evidence twenty

minutes later (fig. 1). They increase in intensity and may but do not always, become more rapid. Each wave is of one to three minutes' duration. When the stomach is nearly empty (as determined by the stomach tube or induced vomiting) they become conspicuous and at this stage of digestion there usually appear superposed upon them, stronger contractions which increase in vigor and are felt by the subject as hunger pains (fig. 2). Although it is by no means always the

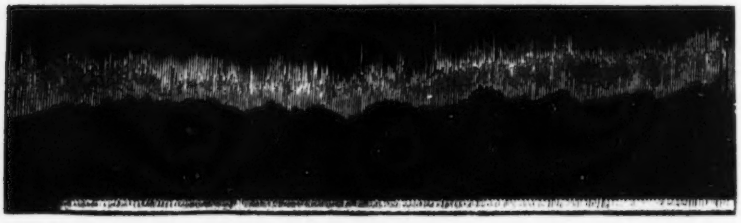

Fig. 1. Tonus rhythm of fundus of stomach (man) 20 minutes after a hearty dinner. Time in seconds.

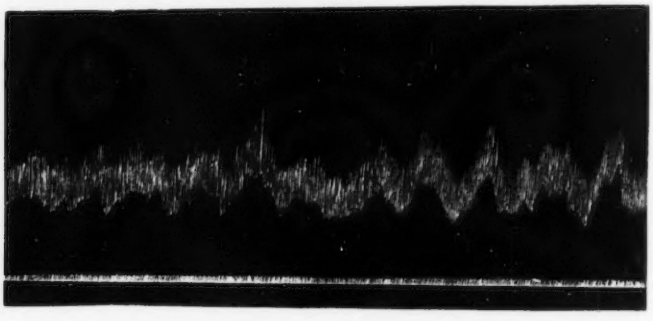

Fig. 2. Tonus rhythm of stomach (man) three hours after dinner (beefsteak, spaghetti, bread, butter, apples and cream).

case, it is significant that the first contractions felt may occur when the stomach still contains traces of food.

Carlson (6) in his first paper describing the hunger contraction noted that the onset of a hunger period was marked by the appearance of a slow tonus rhythm which gradually increased in vigor and culminated in the hunger contractions. As stated above this tonus rhythm is

present not only as an immediate precursor of the hunger period but also throughout the course of normal gastric digestion.

Many workers have employed the rubber balloon in recording the stomach movements but none of the published tracings so far as we are aware, indicate the presence during normal digestion of a slow continuous rhythm as herein described.

Moritz (7) reported contractions of the fundic end of the stomach occurring two and one-half to three and one-half times per minute. According to Dietlan (8) the time required for the peristaltic contractions of the pyloric end of the stomach is twenty to twenty-four seconds. We, therefore, think as suggested by Cannon and Carlson that the tracings of Moritz record the intragastric variations due to the pyloric peristalsis.

Sick (9) using the same method reported that there occur tonus variations of the fundic end of the stomach which give rise to the peristaltic waves. These occur at the rate of two to four per minute in the full or empty stomach. The tracings of Sick show three kinds of pressure variations: Respiratory, cardiac, and "Magen tonus schwangungen," the latter averaging twenty seconds each. The duration of these "stomach tonus variations" coincide with the time intervals required for the pyloric peristalsis.

Is the hunger contraction simply an augmented peristaltic contraction or a contraction of the fundus as a whole? To answer this we studied the stomach movements in the dogs and in ourselves by the "balloon X-ray" method. We were able to make direct observations of the movements of the balloon in the stomach and at the same time note the character of the graphic record.

The upper part of the balloon was held in the cardiac end of the stomach. A young and vigorous dog was employed for the experiments. The dog was starved for intervals of thirty-six to forty-eight hours and in order not to have the hunger contractions completely inhibited by the excitement attendant upon the X-ray examination, frequent repetitions were made until the dog became accustomed to the necessary manipulations. The vigor of the stomach contractions thus made visible to the naked eye is surprising. The weaker type of hunger contractions begin as a constriction in the cardiac end of the stomach and pass down toward the pyloric end as a rapid peristaltic wave. In the very vigorous contraction the wave spreads over the stomach so rapidly that it is difficult to decide whether there is a contraction of the fundus as a whole or a very rapid peristalsis (fig. 3).

Such a condition may well be compared to the peristaltic rush of the intestine as described by Meltzer and Auer (10).

When the X-ray observation of the hunger contractions were made in ourselves, with subject in reclining position a series of photographs were taken of the balloon at times when the record showed the stomach to be comparatively at rest and at varying intervals during a period

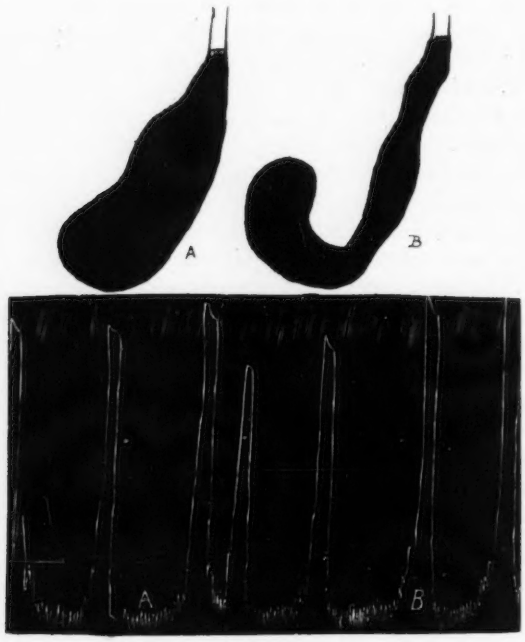

Fig. 3. Hunger contractions of dog's stomach after 30 hours' starvation. *A*, Outline of stomach (as seen by X-ray and bismuth balloon), between hunger contractions with stomach relaxed. *B*, Outline of stomach at the height of a hunger contraction.

of hunger contraction (figs. 4, 5, 6, 7). We made sure the balloon was just below the cardia by pulling the distended balloon upward as far as it would come. By careful observation we were able to tell from the movements of the balloon when a hunger pain was being felt by the subject. In every case the graphic record and the subject

confirmed our own observations. When a hunger pang was felt, a series of constrictions passed rapidly over the balloon. Beginning at the cardiac end, they swept rapidly toward the pyloric end increasing in strength as they proceeded. It was readily seen that the hunger contractions are powerful peristaltic contractions, which, arising at or near the cardiac sphincter, swept downward over the entire stomach. During a typical hunger period the stomach exhibits movements which resemble very closely the movements which have been described by clinicians in patients after a bismuth meal as hyperperistalsis, but described by Cole as normal peristalsis of a stomach that contains small quantities of food. Observations of the hunger contractions of the dog were made after thirty-six hours' deprivation of food, on ourselves after fifteen hours. Prolonged starvation in the case of the dog was necessary to overcome the inhibitory influence of the excitement attendant upon the X-ray examination. Whether or not the stomach of man after a longer period of starvation would show a condition approaching that described for the dog, we are not in a position to state.

The current teaching with reference to the part played by the fundus during digestion is that it is a reservoir, exerting a tonic grasp upon its contents. The kinematographic figures of the stomach published by Kästle, Ridder, and Rosenthal show that the fundus is not quiescent during digestion. Cole (12) has shown that when food is in contact with the cardiac end of the stomach, contractions begin in the fundus and frequently are as deep in this region as in the pylorus. Forsell (13) describes the fundus changes as follows:

> In the standing position, the fundus shows no peristalsis. In the reclining position there occur typical persitalses of the fornix. The fornix empties itself principally by a concentric contraction of the whole wall. At the conclusion of gastric digestion there occur circular contractions of the wall. One may then observe stages in which the fornix assumes a more oval form: then a circular contraction presses the food into the korpus: then a stage of rest in which the fornix takes a more spherical shape. The peristaltic period is begun by a transverse division of the fornix which presses the fornix into a more cylindrical shape and forces the contents into the korpus. This is repeated at intervals until the fornix is empty.

These circular contractions of the stomach may be the cause of the rhythmical pressure variations which we found and which we here speak of as tonus rhythm. That there is a certain degree of independence between the slow tonus rhythm and the hunger contractions is shown by the behavior toward inhibiting agents. Carlson (14) showed

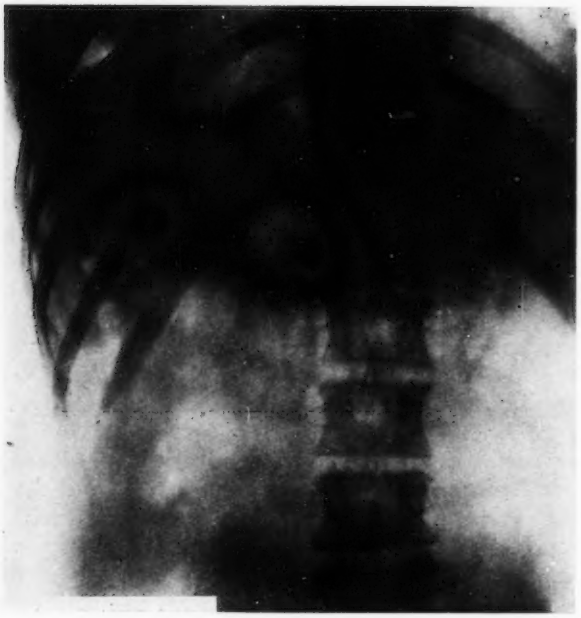

Fig. 4. X-ray photograph (Dr. Rowntree), of the bismuth balloon in the stomach when the graphic record shows the stomach to be quiescent.

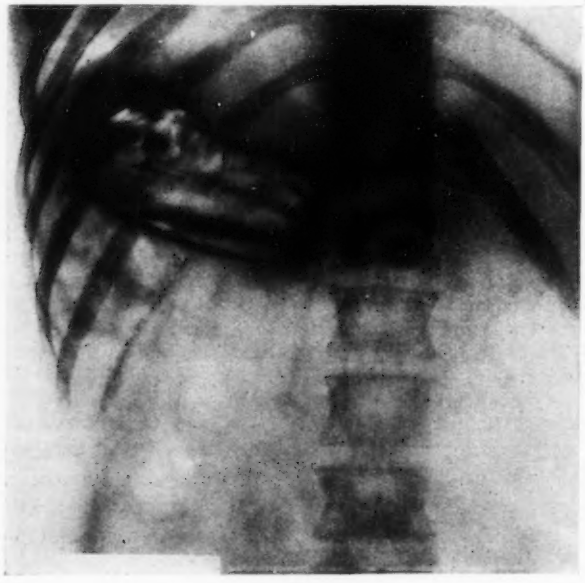

Fig. 5. X-ray photograph (Dr. Rowntree), of the balloon in the stomach at the height of a moderately strong hunger contraction.

Fig. 6. X-ray photograph (Dr. Rowntree), of the balloon in the stomach at the height of a very strong hunger contraction.

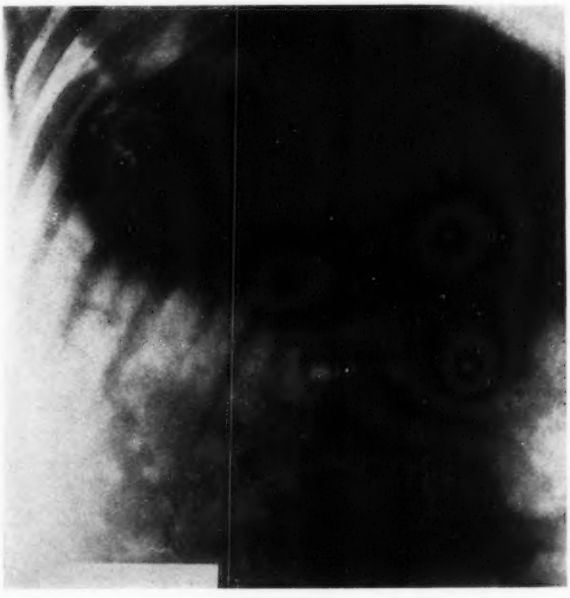

Fig. 7. X-ray photograph (Dr. Rowntree) of the balloon in the stomach near the end of a hunger contraction.

that numerous substances and circumstances inhibit reflexly the hunger contractions. This we found true in ourselves, but this tonus rhythm does not show the same sensitiveness toward the same inhibiting influences. Thus tasting sugar or acid, swallowing water or 0.36 per cent hydrochloric acid solution in small quantities (10—25 cc.) do not inhibit this rhythm or only very slightly (fig. 8). This is in contrast to the immediate effect of the same substances on the hunger contractions.

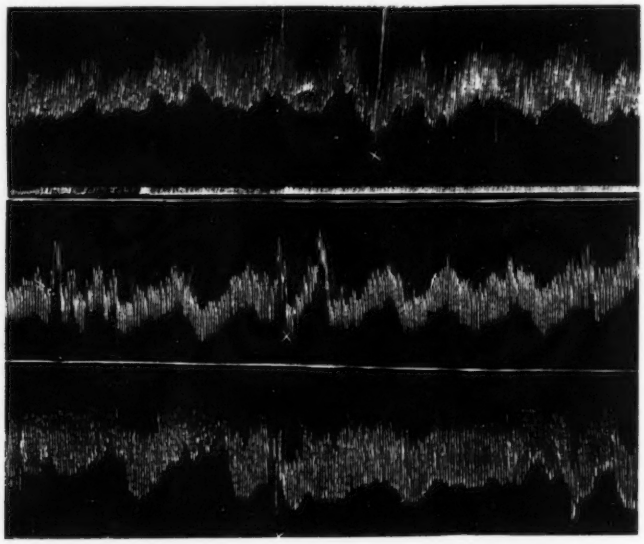

Fig. 8. Tonus rhythm of stomach (man) during gastric digestion is not inhibited to the same degree by substances which completely inhibit the hunger contractions. *A*, at *x* 25 cc. of 0.36 per cent HCl was swallowed. *B*, at *x* 50 cc. of cold water swallowed. *C*, Tonus rhythm just before a hunger period; at *x* 50 cc. of cold water was drunk.

Large quantities of liquids do inhibit the tonus rhythm probably as a result of the distension which a sudden increase in volume of gastric contents would necessitate. A quantity of liquid which does not inhibit the rhythm in a filled stomach may do so in an empty stomach. Excitement, interest, worry or any strong psychic influences immediately inhibit the rhythm. The twenty-second rhythm is not so easily inhibited as either the hunger contractions or the slower tonus rhythm.

Our findings interpreted in the light of the contributions of Cannon, Carlson and Forsell show that the normal stomach exhibits the following types of muscular activities:

1. A tonic grasp of the upper stomach musculature upon the food. This tonic condition exhibits slow rhythmical variations.

2. Peristaltic contractions of the antrum pylori.

3. Peristaltic contractions of the entire stomach (the hunger contractions of Boldyreff, Cannon and Washburne, and the thirty-second rhythm of Carlson).

4. In the young dog the vigorous hunger contraction begins with a constriction at the cardiac end and is followed by a contraction of the entire fundus. (If this type of contraction be peristaltic the waves are too rapid to be detected by our methods.)

During normal digestion the peristaltic waves sweep over the lower part of the stomach. The fundus exhibits a rhythm of the same rate as was shown by the work of Carlson and Orr. In the meantime there are slow rhythmical tonus variations of the upper part of the stomach. As the stomach empties itself the peristaltic waves arise from points higher and higher toward the cardiac end of the stomach run over the entire stomach, culminating in a more or less tetanic contractions of the antrum. The hunger contractions are therefore strong peristaltic contractions in a stomach whose condition compared with its normally filled condition is hypertonic. Evidently these two phases of stomach contractions are most intimately related. The tonus may become so great as to run into tetanus or it may maintain a level on which the separate peristalses are superposed. These two different activities are not necessarily similarly affected by the same inhibiting influences. Both may be inhibited by introducing food or liquid into the mouth or stomach or by psychic influences but not to the same degree.

CONCLUSION

1. Both in man and in the dog, the fundic end of the stomach, during normal digestion, exhibits a slow tonus rhythm of one to three minutes' duration. During the emptying of the stomach and the onset of the hunger period, this tonus rhythm becomes more vigorous.

2. In the dog after thirty-six hours' starvation the stomach is markedly hypertonic; a vigorous hunger contraction is a contraction of the fundus as a whole.

3. In man after fifteen hours' starvation the hunger contractions

are vigorous peristaltic contractions in groups of 2–4, superposed upon the slow tonus rhythm. They begin at the cardiac end of the stomach and sweep over the whole stomach.

4. A given inhibiting agent does not necessarily have the same effect upon the different types of gastric contractions; thus the pyloric peristalsis is the most resistant to reflex inhibiting influences; fundic peristalsis is least resistant, while the tonus rhythm occupies a position between the two.

We wish to express our thanks to Dr. Rowntree of the Presbyterian Hospital under whose supervision the X-ray work on ourselves was carried out, and to Dr. A. J. Carlson for his help and criticism.

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A CONTRIBUTION TO THE PHYSIOLOGY OF LACTATION

W. L. GAINES

From the Hull Physiological Laboratory, University of Chicago

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FOREWORD

The development and functioning of the mammary gland in the female mammal is closely associated with, and dependent upon the reproductive function.[1] The normal course of activity of the gland is somewhat as follows.

Embryonic— The first sign of the mammary organs, the milk line, appears at an early stage in embryonic life, and growth is continued until the gland reaches a considerable development at

Birth— At birth a milky fluid may be expressed from the gland in either sex, indicating a secretory activity, but this activity soon ceases and the gland remains quiescent until

Puberty— At puberty under the influence of the ovaries growth is resumed and carried to a greater or less development, varying with the individual and the occurrence of

Pregnancy— Pregnancy induces a great hypertrophy of the gland, and it reaches a high state of development, accompanied by the accumulation of colostrum, by the time of

Delivery— At this time milk secretion proceeds actively and if the milk is removed at short intervals, by nursing or artificially, secretion continues for a period of days, months, or years. The rate of secretion, however, after a time gradually decreases to zero. This decrease is favored by a succeeding

Pregnancy— Further growth of the gland ensues but not as marked as in the preceding gestation, and at

Delivery— milk secretion is again actively resumed. Whence, the pregnancy-delivery cycle is repeated.

[1] The mammary gland may, in turn, influence the reproductive and other body functions, although such influence is, apparently, not so fundamental.

This paper presents some data and discussion based on an investigation of certain phases of the above outlined course of activity of the mammary gland carried out at the Hull Physiological Laboratory of the University of Chicago. I wish to acknowledge especially the assistance and suggestions of Dr. Carlson throughout the work. I am also indebted to Dr. Mathews and Dr. Koch, among others, for various suggestions.

EXPERIMENTAL METHODS AND RESULTS

Prenatal development. The milk lines may be distinguished as early as the 4 mm. stage in the human embryo, according to Strahl (1) Knoepfelmacher (2) states that in children born prematurely there is no appearance of milk in the mammary glands. In a post mortem examination of two kids, the mother of which died about two weeks before term, I found mammary tissue plainly developed but no appearance of milk in the transected gland. Chemical examination for the presence of sugar was not made and this test might have shown the presence of milk in amount too small to be distinguished by simple cutting of the gland. The udder of the mother, a multipara, was at the time undergoing a rapid hypertrophy as evidenced by increase in size. (Data on this point will be found in table 1, Goat No. 4). A part of the increase in size was due to the accumulation of colostrum, which exuded freely from the cut ducts of the gland after death.

The influence of pregnancy on the growth of the mammary gland. The growth of the mammary gland during gestation is a very striking phenomenon. This development is in preparation for its function of milk secretion following delivery. Ribbert (3) transplanted the mammary gland of a guinea pig into the skin back of the ear. Five months later (the gestation period is about two months) living young were born, whereupon the transplanted gland functioned promptly. Ribbert states that hypertrophy was not sufficiently marked to make the gland apparent beneath the skin, that the nipple did not develop, and there was, therefore, no outlet for the secretion, but that milk was present in the gland. Marshall (4) cites the case of the Bohemian pygopagous twins, Rosa-Josepha, one of whom became pregnant. The breasts of both underwent development during pregnancy and functioned following delivery. Lane-Claypon and Starling (5) produced a development of the mammary glands in a virgin rabbit closely resembling that occurring during pregnancy by means of injecting extracts of rabbit foetus.

On the other hand, Lombroso and Bolaffio (6) using parabiotic methods with rats found no evidence that the pregnancy of one of a pair of females produced any effect on the mammary glands of the other one of the pair. Knoepfelmacher (2) injected blood serum from a pregnant goat into a non-lactating goat. The amounts used were 55, 33, and 90 cc. taken respectively 6 days, 2 days, preceding delivery, and 1 day following delivery. The results were negative.

I have injected defibrinated blood from a pregnant non-lactating, multiparous goat into a non-pregnant, non-lactating, multiparous goat with negative results with reference to increase in the volume of the mammary glands or milk secretion. The volume of the udder was determined by pressing a suitable vessel containing warm water up

TABLE 1

Showing the effect on the volume of the udder of a non-pregnant goat from the intravenous and intraperitoneal injection of defibrinated blood from a pregnant goat

GOAT NO. 4—DONOR (PREGNANT, NON-LACTATING, MULTIPAROUS)		GOAT NO. 3—RECIPIENT (NON-PREGNANT, NON-LACTATING, MULTIPAROUS)		
Date	Volume of udder	Volume of udder	Blood injected	Method of injection
1915	cc.	cc.	cc.	
1-21	935	170	90	Intraperitoneal
1-23	935	165	115	Intraperitoneal
1-25	950	175	100	Intraperitoneal
1-27	980	175	75	Intraperitoneal
1-30	1005	160	15	Intravenous
2-3	1060	170	85	Intraperitoneal
2-6	1090	170	35	Intraperitoneal

around the udder and firmly against the body wall. The amount of water displaced (determined by the difference between the amount left in the vessel and its capacity) was taken to represent the volume of the glands. The method is not exact but is sufficiently accurate to show any marked changes in volume. The data are given in table 1. No appearance of milk resulted during the period of injections or following.

The figures in table 1 show that the udder of the donor was increasing steadily in size but the amount of blood injected failed to affect the size of the udder of the recipient. The latter at a previous time during a similar stage of pregnancy as represented by the donor in the present instance had fully as large an udder as the donor. The figures, then

serve to show the enormous difference in size of the udder with the stage of pregnancy.

The influence of pregnancy on milk secretion. Lane-Claypon and Starling (5) advance the theory in connection with the work referred

TABLE 2

Daily milk and fat yield of goat No. 2, showing the effect of intravenous injections of defibrinated blood from a pregnant goat, No. 3; from her two days old kid; and again from No. 3 six days after delivery. No. 2 said to have dropped her kids about April 12. No. 3 kidded August 8, 1914

DATE	MILK	FAT	FAT	REMARKS
1914	*cc.*	*per cent*	*gms.*	
7-24	823	5.1	41.9	
7-25	796	6.4	51.0	
7-26	730	6.4	46.8	
7-27	645	5.4	35.0	
7-28	720	6.4	46.3	Injected intravenously 175 cc. defibrinated blood
7-29	**463**	**6.9**	**31.9**	from No. 3 (10 days before term)
7-30	708	6.8	48.2	
7-31	674	6.2	41.7	
8-1	783	6.8	53.7	Injected intravenously 300 cc. defibrinated blood
8-2	**552**	**7.0**	**38.6**	from No. 3 (7 days before term)
8-3	783	7.0	54.8	
8-4	847	7.8	66.1	Injected intravenously 175 cc. defibrinated blood
8-5	913	7.5	68.4	from No. 3 (3 days before term). Caused some
8-6	**349**	**4.9**	**17.1**	general depression, weather hot, flies trouble-
8-7	633	8.0	50.8	some
8-8	832	6.2	51.6	
8-9	752	5.5	41.1	
8-10	665	5.9	39.3	Injected intravenously 100 cc. defibrinated blood
8-11	**538**	**6.6**	**35.4**	from male kid of No. 3. Age of kid 2 days
8-12	806	6.8	54.7	
8-13	873	6.0	52.4	
8-14	853	6.5	55.0	Injected intravenously 100 cc. defibrinated blood
8-15	**765**	**7.0**	**53.7**	from No. 3 (6 days after delivery)
8-16	880	6.3	55.4	
8-17	823	7.3	59.6	
8-18	848	6.2	52.3	

to above that the developing foetus passes a substance into the maternal circulation which favors the growth of the mammary gland but inhibits its secretory activity. The removal of this inhibitor, then, at delivery allows the gland to function in its milk secretory capacity.

Bearing on this point I have made the following series of injections

of defibrinated blood into a lactating goat: 1, from a pregnant goat near term; 2, from a kid of this same goat 2 days after birth; 3, from the same goat 6 days after delivery. Data of this test are given in table 2.

The figures indicate an inhibitory action from each of the transfusions. The milk and fat data as given for each day are the sum of two milkings made throughout at approximately 12-hour intervals. In every case the inhibitory influence was most marked at the first milking following the transfusion. Except in the case of the transfusion made August 5 there was no apparent systemic disturbance

TABLE 3

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-freed cow's placenta; also, defibrinated blood from goat No. 5. No. 6 said to have kidded March 12, 1915. No. 5 dropped kids April 17, 1915, and at the time of transfusion, April 24, was milking around 2000 cc. per day

DATE	MILK	FAT	FAT	REMARKS
1915	*cc.*	*per cent*	*gms.*	
4–18	475	6.8	32.3	
4–19	488	7.3	35.5	
4–20	460	6.4	29.3	Injected intraperitoneally a water extract of 5 gms. of dried, fat-freed cow's placenta
4–21	**168**	**9.4**	**15.8**	
4–22	238	8.5	20.4	
4–23	365	6.7	24.4	
4–24	315	5.9	18.5	Injected intraperitoneally 200 cc. defibrinated blood from No. 5 (fresh 7 days and giving about 2000 cc. daily)
4–25	**182**	**8.6**	**15.7**	
4–26	**147**	**7.1**	**10.5**	
4–27	**111**	**9.4**	**10.4**	
4–28	183	7.8	14.3	
4–29	154	8.3	12.8	

in the condition of the recipient. Leaving this case out of consideration the inhibitory action seems to be equally marked for either the blood of the pregnant goat or that of her two-day old kid.

The transfusion made 6 days after delivery had only a very slight inhibitory action as seen in the yield of milk and was not at all reflected in the yield of fat. Results in another trial with two other goats were similar to the above except in the transfusion following delivery (see table 3).

Effect of placental extract on milk secretion. Niklas (7) found that an extract of placenta caused milk production in virgins and mothers.

Lederer and Pribram (8) found greatly increased secretion of milk upon intravenous injection of placental extract.

Table 3 presents the data of a test of the effect of an extract of cow's placenta on milk secretion in the goat. The fresh placental tissue was ground, dried, extracted with gasoline and ether to rid of fat, and the residue extracted with water as needed. The figures show a distinct inhibition, quite similar in nature to that just observed from the blood of a pregnant goat or new-born kid (see table 2).

Relation of the blood to milk secretion. The activity of the mammary gland with reference to milk secretion varies enormously. A large part of the time it may be at zero, but immediately following a normal pregnancy and delivery it is at a high point. To test for the presence of a substance in the circulating blood stream which accelerates the secretory function of the mammary gland during the period of its highest activity following delivery, transfusions were made from a fresh, heavy milking goat to one giving a low yield. No increase in secretion was obtained by this means. In table 2 no change, or a very slight decrease was shown. In table 3 there is shown a very marked decrease. In this latter case the donor was milking heavily at the rate of about 2000 cc. daily, including the milk taken by two nursing kids. The recipient although giving a relatively low yield, about 300 cc. daily, had been fresh only about 6 weeks and would seem to have offered a favorable subject for positive results. No general disturbance was apparent to account for the decrease noted.

The effect of mammary gland extract on milk secretion. Gavin (9) fed mammary gland preparations to cows without affecting the yield or quality of the milk. The mammary gland of the same cow whose placenta was used in the data shown in table, 3, was prepared in the same way as given for the placenta. Results from the injection of water extracts of this material are given in table 4.

The figures appear to show a temporary inhibition followed by an increase to something above the previous yield. The net result of the several injections seems to be a considerable increase, but it may be that this should not be attributed to the injected material. Reference to table 3 shows that goat No. 6 was, a few days before, giving more than the yields recorded in table 4, and the apparent increase may be nothing more than recovery following the blood transfusion of April 24. However, the immediate inhibition, similar to that noted for the placental extract, seems to be unequivocal.

The action of pituitrin on the mammary gland. Since Ott and Scott (10) first noted that pituitrin had a galactagogue action this material has received considerable attention in its relation to milk secretion. All reports of investigators on the immediate action of pituitrin confirm the observation of Ott and Scott, viz., that its injection into the circulation causes an immediate flow of milk in a lactating animal. MacKenzie (11) working with cats in anesthesia and observing the flow of milk from the cut surface of the gland found pituitrin the most active of the several animal extracts he studied. Gavin (9) working with

TABLE 4

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-freed cow's mammary gland (pregnant). No. 6 said to have dropped kids March 12, 1915

DATE	MILK	FAT	FAT	REMARKS
1915	*cc.*	*per cent*	*gms.*	
5–3	113	8.3	9.4	
5–4	85	6.7	5.7	
5–5	90	7.7	6.9	
5–6	119	7.6	9.0	Injected intraperitoneally water extract of 5 gms.
5–7	**75**	**6.5**	**4.9**	cow's mammary gland
5–8	135	6.2	8.4	
5–9	143	6.6	9.5	
5–10	176	6.4	11.2	Injected intraperitoneally water extract of 1 gm.
5–11	**157**	**7.1**	**11.1**	cow's mammary gland
5–12	190	6.9	13.1	Injected intraperitoneally water extract of 4 gms.
5–13	**134**	**7.8**	**10.4**	cow's mammary gland
5–14	189	7.4	13.9	
5–15	220	7.5	16.4	Injected intraperitoneally water extract of 5 gms.
5–16	**177**	**7.7**	**13.7**	cow's mammary gland
5–17	194	7.3	14.3	

cows yielding 15–35 pounds of milk daily found no effect on quantity or quality of milk as the average result of 3–5 days treatment with pituitrin. In some cases, he reports, there was a distension of teat and cistern with milk immediately following injection. Hill and Simpson (12) report similar results. Hammond (13) has studied in some detail the effect of pituitrin on the composition of milk secreted under its influence. He finds that the fat content is greatly increased and that the protein, sugar and ash content remain very constant. This refers to the milk secured immediately after the injection of pituitrin and is not in conflict with Gavin's results which referred to longer periods;

for the yield of milk and its fat content are later depressed. Hammond concludes from his data that the action of pituitrin is not muscular. Hill and Simpson (14) confirm Hammond's results as to the composition of milk obtained after the use of pituitrin except they did not find the depression in fat content later, as noted by Hammond.

Heaney (15) found in the human a contraction of the breast upon injection of pituitrin followed by a return to initial volume, and attributes the milk secretory action of pituitrin to its muscular effect.

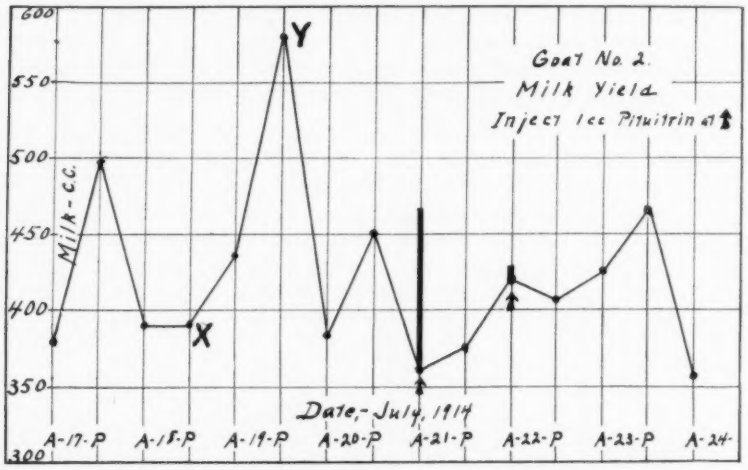

Fig. 1. Showing fluctuation in yield of milk by a goat milked at regular 12-hour intervals and the dependence of the flow of milk produced by the injection of pituitrin upon this fluctuation. The heavy vertical lines represent the yield of milk obtained upon intravenous injection of 1 cc. of pituitrin immediately after the regular milking. It is inferred, for example, that pituitrin would have caused a large further flow at *X*; while at *Y* little or no extra milk would have been obtained. *A* = morning milking; *P* = evening milking.

As to the quantity of milk and fat yielded as a result of the use of pituitrin (Parke & Davis preparation was used throughout the work here reported on pituitrin) based on the average of two days and not the immediate yield, my data show no effect either way. My results, based on three goats, confirm those of Hammond who found a decrease in fat content of the milk for a day or two following the use of pituitrin. I have found no marked or consistent variations in the sugar or protein content of the milk.

The flow of milk from an active mammary gland, which has not been drained for a few hours, upon injection of pituitrin is a very striking reaction. In the goat I have never failed to secure some flow of milk from its use even immediately after milking "dry." The amount obtained, however, varies considerably.

Figure 1 illustrates this point. The goat was milked regularly at 12-hour intervals but the yield is seen to fluctuate widely. The intravenous injection of pituitrin immediately following a regular milking produces a further flow varying in amount inversely with the yield secured by the hand milking which preceded it. That is, injection of pituitrin following a relatively high yield of milk produces a small flow; and following a relatively low yield it produces a large flow. A second, third or fourth dose produces only a very slight flow, 2–4 cc. in the goat.

I have followed the yield of milk in the nursing dog very satisfactorily by keeping the pups separated from the mother and allowing them to nurse at 8-hour intervals. The pups were balanced on a smooth-working balance, with shot, then allowed to nurse, and again balanced with the standard weights. The increase in weight represents approximately the yield of milk by the mother.

Under the above conditions, after the pups have nursed fully (6 or 7 minutes), I have never succeeded in producing any further flow of milk by the intravenous injection of pituitrin. But if the mother be placed under ether at the regular nursing hour and the pups then allowed to nurse the yield of milk is greatly depressed, although the pups do their part of the act in the usual vigorous manner. Often, in fact usually, the yield is so low as not to be detectable by the method used (that is, it appears, well under 5 gms., considering errors in loss of saliva, etc.). If, now, pituitrin be injected intravenously, with the mother still under ether, the pups immediately secure the normal yield, or the balance of it which they had failed to get in the first nursing, and this, in rather less time than is required in nursing under normal conditions. A second dose following the first in 10 minutes, produces no further flow.

Figure 2 shows an illustration of the effect of pituitrin under normal conditions and also with the mother under ether, but in the latter case some milk yielded to the pups nevertheless. Ordinarily the ether curve would hold to the base line until the pituitrin was administered.

While pituitrin causes no further flow of milk in the dog after nursing, this does not seem to hold true for the goat. Data from two

experiments are given in table 5 which show that after the kid had nursed and secured all the milk it could, injection of pituitrin still caused a further marked flow. The high fat content (15.8 to 19.6 per cent) of the milk thus obtained is also remarkable.

Bearing on the nature of the mechanism involved in the flow of milk produced by pituitrin under certain conditions, the following test was carried out. A cannula was inserted in the teat of a goat and connected with a chloroform manometer recording on the revolving drum of a kymograph. The gland was then inflated with air through a T-

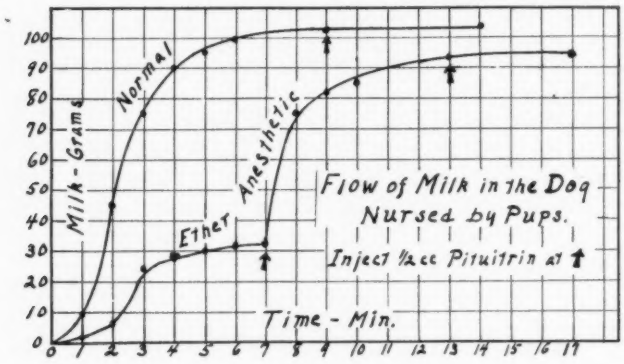

Fig. 2. Showing failure of pituitrin to cause a flow of milk following normal nursing in the dog; and, its restoral of the flow normal to the stimulus of nursing when the normal flow is inhibited by anesthesia; and, the failure of a second dose to cause any further flow. Also, showing under the stimulus of normal nursing the latent period in the flow of milk during the first minute, followed by the very rapid flow for the next two minutes, and then the gradual decline to zero (cf. figs. 4–8).

connection to a pressure of 8–10 cm. chloroform. The device may be made quite sensitive in recording any change in pressure within the gland. With the apparatus properly adjusted pituitrin was injected intravenously. The effect varies with the stage of activity of the gland. In the non-pregnant, non-lactating goat there is no apparent effect. This is true, also, in the pregnant, non-lactating goat up to a time close to term. A short time before term the result, following a latent period of 15–20 seconds, is a slight increase in pressure with a return to the initial. In the freely lactating goat, after a latent period

of 15–20 seconds, there is an abrupt rise in pressure followed by a gradual and nearly uniform decline to the initial pressure. The decline occupies about 5 minutes. A series of 3 curves, taken from the same goat before and after delivery, is given in figure 3.

A second dose of pituitrin in the freely lactating goat gives a curve similar to the first dose, except that it is much reduced in the extent of the rise. In the case of a goat milking from one gland and dry in the other, each gland being separately connected as above, pituitrin

TABLE 5

Showing the effect in the goat of nursing following milking "dry" by hand; and of pituitrin following nursing. After milking by hand, kid nursed left gland and at the same time right gland was milked by hand. Following the nursing 1 cc. pituitrin was injected intravenously and both glands milked by hand. Goat No. 5, kidded April 17, 1915

EXPLANATION	RIGHT GLAND			LEFT GLAND			BOTH GLANDS		
	Milk	Fat	Fat	Milk	Fat	Fat	Milk	Fat	Fat
	cc.	*per cent*	*gms.*	*cc.*	*per cent*	*gms.*	*cc.*	*per cent*	*gms.*
4–22–15									
Milked "dry" by hand							358	3.5	12.5
Nursed by kid				153					
Milked by hand	**190**	**9.8**	**18.6**				343		
Injected pituitrin—milked by hand	70	15.8	11.1	98	17.1	16.8	**168**	**16.6**	**27.9**
5–2–15									
Milked "dry" by hand							315	4.7	14.8
Nursed by kid				175					
Milked by hand	**155**	**13.3**	**20.6**				330		
Injected pituitrin—milked by hand	45	19.1	8.6	40	19.6	7.8	**85**	**19.3**	**16.4**

produced the typical curve in each gland, that is, the abrupt rise and slow decline in the active gland, and no change in the non-lactating gland.

While the above test indicates a contraction of the ducts and alveoli of the gland with a subsequent relaxation, it does not definitely localize the seat of the contraction. That is, the effect might be produced by the activity of the muscles of the large ducts. Indirect evidence on the point is afforded by the following test.

A lactating guinea pig was separated from her nurslings for two hours to allow the accumulation of milk in the glands. The animal was then anesthetised and a mammary gland dissected entirely away from the body with as little injury to the gland as possible. The end of the nipple was cut off to allow free egress to any milk. A few drops of pituitrin were now injected into the gland substance, whereupon there was a copious flow of milk from the cut nipple. A control injection of salt solution gave no such effect.

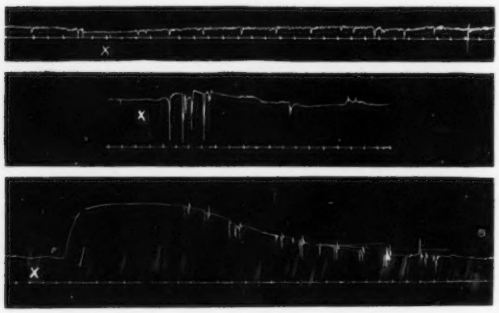

Fig. 3. Showing the effect of pituitrin on the pressure of an air-inflated mammary gland of a goat. The teat was connected with a cannula and tube to a chloroform manometer and the gland inflated to a pressure of 8–10 cm. At *X* ¾ cc. of pituitrin was injected intravenously. The time line marks 10-second intervals. The upper tracing was taken 37 days before delivery; the middle tracing, 17 days before delivery; the lower tracing, 12 days after delivery (the daily milk yield at that time being around 1800 cc.). The shallow waves, seen especially in the upper tracing, are due to respiratory movements. The deep, sharp irregularities are due to bleating and struggling of the animal.

A similar gland preparation was cut across with a razor, exposing a cut surface as remote as possible from the large ducts and vessels. A drop of pituitrin was placed on the cut surface and in a few seconds many minute white dots appeared beneath the pituitrin and slowly swelled to tiny milky rivulets streaming beautifully through the clear liquid. Similar preparations from the cat and dog gave like results.

The reflex involved in milking and nursing. According to Flower and Lydekker (16) the mother whale ejects the milk from her mammary glands into the mouth of the young without the active sucking on the part of the young which is generally common to mammals. Schäfer (17) states that the discharge of milk during the act of sucking or milking is in part the result of direct mechanical pressure upon the milk reservoirs of the larger ducts; and partly due to a contraction of the plain muscular tissue which accompanies these ducts, and which appears to

be set in action by mechanical stimulation of the nipple. He adds that the flow is probably aided by the swelling of the gland due to a reflex dilatation of its arterioles.

The curve of normal nursing in the dog given in figure 2 shows roughly the rate at which the pups secured milk during the process of nursing. The data on which the curve is based were obtained by working the pups in two relays of three pups each. Group I was allowed to nurse for 1 minute, then removed and Group II given way for 1 minute, Group I being meanwhile weighed. Places were then again exchanged and so on until they had obtained all the milk possible.

The normal curve shows that the pups could secure only a small amount of milk the first minute, but during the second and third minutes there was a relatively large amount of milk available to their

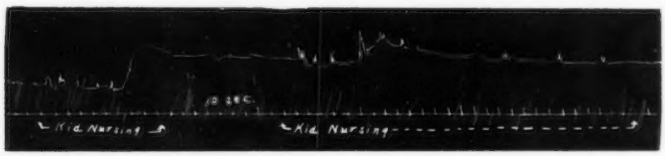

Fig. 4. Showing the pressure changes produced in one gland under the influence of kid nursing the other gland. Goat No. 5, fresh 10 days, milked 3 hours previously. Left gland connected with chloroform manometer (no inflation of gland). Kid nursing right gland. The sharp irregularities in the curve are due to bunting of the kid. Time line marks 10-second intervals and represents 7 cm. pressure (cf. figs. 5, 6, 7, 8).

efforts. After the third minute the amount available declines and at the end of 6 minutes the total supply appears to be exhausted. The very marked changes in the amount of milk secured with the progress of nursing are not attributable to any differences in the efforts of the pups themselves to secure their food.

In table 5 it is shown that, after milking the mother goat as completely as possible by hand, if a kid be allowed to nurse one gland he not only obtains a considerable quantity of milk but there is also a practically equal flow in the other gland which may now be readily obtained by hand milking. The full significance of this reaction with reference to the operation of a reflex contraction and ejection of milk from the gland is perhaps better given by the records of the following tests.

I. Change in pressure. One gland was connected by a cannula and tube to a chloroform manometer recording on a kymograph. A kid

was then allowed to nurse the other gland. An example of the effect is given in figure 4. The pressure registered upon insertion of the cannula was about 9 cm. During the period when the kid was nursing the curve shows a latent period of 60 seconds with no change in pressure aside from the purely mechanical effect of the impatient bunting of the kid. At the end of this latent period there appears a marked rise in pressure. That, coincident in time with this rise in pressure, the kid was obtaining a satisfactory supply of milk, was plainly evident from the cessation of his bunting (shown, as well, in the smoothness of the curve), his rapid guzzling, and the rounding out of his little paunch. Two minutes later a second kid was allowed to nurse and a rise in pressure occurred; again only after a latent period of about 1 minute.

II. Flow of milk. A cannula was inserted in one teat and the milk flowing through it led by a short tube to one limb of a U-tube of large bore. The U-tube was held in a vertical position and its other limb was provided with a float and rod recording on a kymograph after the usual fashion of a manometer. Enough water was put in the U-tube to allow the float to ride freely, so that the addition of any further liquid would be properly registered. The connection of the tube from the teat with the U-tube was open so that the liquid in both limbs was constantly under atmospheric pressure only. The outlet for the milk remained constantly about 10 cm. below the level of the teat. With the apparatus adjusted a kid was allowed to nurse the free teat. Examples of the results are given in figures 5–7.

In the experiment represented by figure 5 the right gland was connected as described above and the left gland milked by hand. After the hand milking was completed a kid was allowed to nurse the left teat. During the first 35 seconds of the kid's nursing the curve shows a flow amounting to a few drops only from the right gland. At the end of a 35-second latent period it shows a rush of milk reaching a maximum in 20 seconds and being practically complete in 80 seconds. The form of this curve is strikingly similar to that of the curve of flow in normal nursing of the dog shown in figure 2.

The high fat content (13.3 per cent) of the milk obtained under the stimulus of the nursing of the kid is noteworthy and significant. The amount of fat contained in the second portion was 50 per cent greater than that obtained under the stimulus of the first milking operation.

In the trial shown in figure 6 preliminary hand milking was not used. Fifteen cc. of milk flowed upon insertion of the cannula in the course of 3 minutes. Upon nursing of the opposite gland by the kid there is

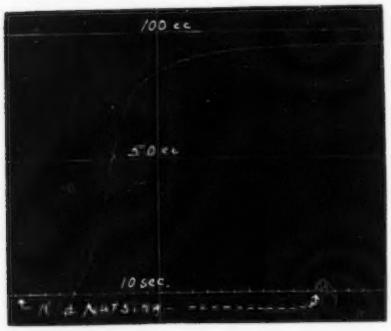

Fig. 5. Showing flow of milk through cannula from one gland under the stimulus of kid nursing the other gland. Goat No. 5, fresh 24 days, milked 11 hours previously. Cannula inserted in right teat and led to instrument recording the flow of milk. 84 cc. of milk flowed immediately on insertion of cannula. Left gland then milked as dry as possible by hand, yielding 136 cc. 45 seconds after the commencement of hand milking a further flow started from the right gland, yielding in 2 min. 68 cc. (results to this point not shown in the figure). Kid then nursed left gland causing a rapid flow from the right gland, after a latent period of 35 seconds as shown (cf. figs. 2, 4, 6, 7, 8).

		Milk, *cc.*	Fat, *per cent*	Fat, *gms.*
I	Left gland, hand milked	136	5.6	7.6
	Right gland, cannula flow	152	5.3	8.1
II	Left gland, kid nursed	90		
	Right gland, cannula flow	95	13.3	12.6

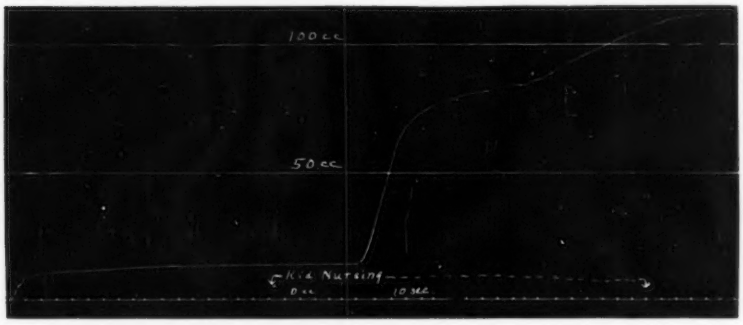

Fig. 6. Showing same as figure 5. Goat No. 5, fresh 11 days, milked 3½ hours previously. Left gland connected with flow recorder, kid nursed right gland (no hand milking). Initial flow on insertion of cannula, 15 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 100 cc. Total flow 115 cc., 7.1 per cent fat (cf. figs. 2, 4, 5, 7, 8).

shown a latent period of 60 seconds and then a rapid flow from the unnursed gland. A second marked increase in flow is shown to occur 2 minutes following the first ejection. The curve shows, in comparison with figure 5, first, a longer latent period, and second, the occurrence of a second ejection of milk. In some cases the curve takes the form of 4 or 5 distinct steps.

The conditions of the test shown in figure 7 are practically identical with those of figure 6. The curve is given as showing another type of reaction, viz., a protracted and more or less uniform flow of milk (although a tendency to steps in the curve is shown here, also) under the stimulus of the kid's nursing. That the kid was also experiencing delay in the speed with which he was served his meal is evidenced by the length of time he continued to nurse.

Fig. 7. Showing same as figure 5. Goat No. 5, fresh 16 days, milked 4 hours previously. Left gland connected with flow recorder. Kid nursed right gland (no hand milking). Initial flow on insertion of cannula, 7 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 44 cc. (cf. figs. 2, 4, 5, 6, 8).

Figure 8 brings out two points: (1) The curve of milk flow with the simple insertion of a cannula and without any other manipulation may take a distinct step form, as shown in the left hand part of the figure. (2) Hand milking of one gland produces a flow in the opposite gland, similar to the effect noted for the kid's nursing. (Other trials gave higher and steeper curves, very like that of figure 6.) The latent period is distinct, being 55 seconds in the present instance.

The question arises as to the nature of the factor or factors involved that cause the further ejection of milk upon nursing of the kid following hand milking, and which it seems to be impossible to call into play by usual methods of milking. I have tried various methods of electrical stimulation without success. I have imitated the nursing action

of the kid as actively as I could with my own mouth for several minutes at a time without result in the way of a further milk flow. The presence of the kid, nosing about the udder but not allowed to take a teat into his mouth, while using the greatest possible persuasiveness in the art of dry-hand milking was not sufficient. Neither did it suffice to add to the last mentioned recourse conditions of moisture and temperature by immersing the teat in water at 38–40°C. Only by allowing the kid to nose about the udder, while applying my own mouth to a teat in

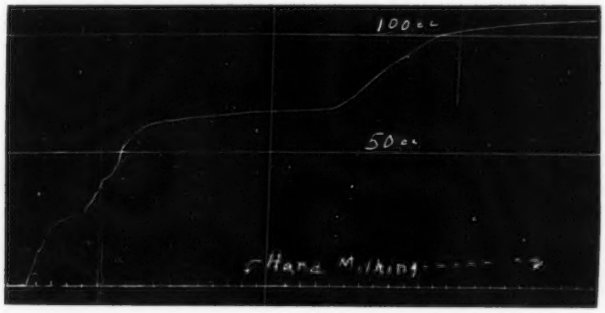

Fig. 8. Showing flow of milk through cannula from one gland under stimulus of hand milking of other gland. Goat No. 5, fresh 23 days, milked 12 hours previously. Left gland connected with flow recorder. Initial flow on insertion of cannula, 27 cc., followed by second flow of 44 cc. Flow under influence of hand milking other gland, after latent period of 55 seconds, 33 cc. Total flow 104 cc. Kid then nursed right gland obtaining 140 cc. and causing a further flow from left gland of 124 cc. (not shown in the figure), (cf. figs. 2, 4, 5, 6, 7).

		Milk *cc.*	Fat *per cent*	Fat *gms.*
I	Right gland, hand milked	136	3.6	4.9
	Left gland, cannula flow	104	3.3	3.4
II	Right gland, kid nursed	140		
	Left gland, cannula flow	124	9.3	11.5

imitation of his sucking, have I been able to bring about the desired reaction without the actual nursing of the kid. The following protocol illustrates.

Goat No. 5. 5/16/15, 7.15–7.45 p.m.

	Milk	Fat	Fat
	cc.	*per cent*	*gms.*
Milked by hand in usual manner (kid not around).....	285	7.9	22.5
Sucked teat with mouth, then milked by hand.....	00	00	00
Kid introduced and attempting to nurse but not allowed to take teat in mouth, then milked by hand.....	00	00	00
Sucked teat with mouth (no milk drawn from teat), with kid present but prevented from nursing, then milked by hand.....	160	17.3	27.7
Kid nursed vigorously and secured.....	3		

The second flow of milk induced by the treatment outlined is not quite as great as in the data given in connection with figures 5 and 8, and table 5, but the rise in fat content is greater, and, in this respect, is much the same as the effect noted from the use of pituitrin given in table 5.

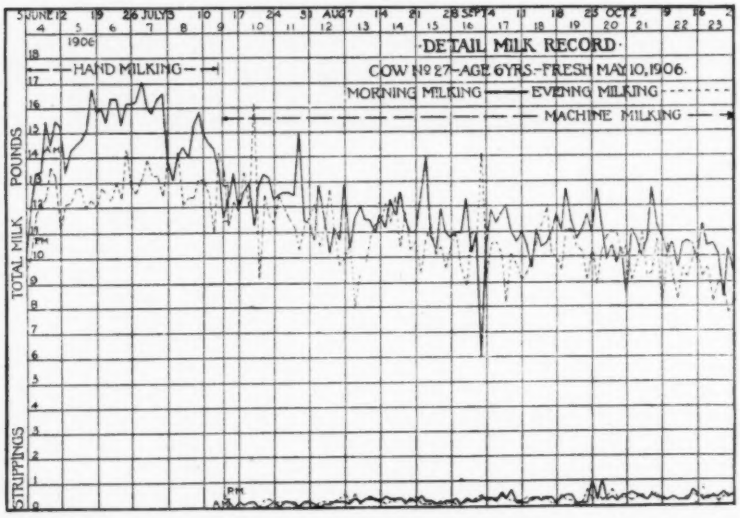

Fig. 9. Showing an illustration of the failure of a milking machine, followed by hand stripping, to secure a uniform degree of removal of the milk from the udder in the cow. Figure from M. S. thesis of the writer, University of Illinois, 1910.

While hand milking does not remove all the milk from the udder that it may be possible to obtain by the use of some stronger stimulus, it usually secures a fairly uniform degree of removal. Thus, in figure

9 showing the yield of milk by a cow after a 13-hour period (morning milking) and after an 11-hour period (evening milking) it is seen that, with hand milking, the curve for the longer period is always above the curve for the shorter period and the two do not cross. However, when machine milking commences, even though it was followed by hand stripping, the character of the curves changes and they cross and intercross repeatedly. This means that the stimulus provided by the machine work did not produce a uniform result. The total yield over a period of some months, it appears, need not be decreased by this condition.

Change in volume of the udder in milking. That the udder of the goat and cow decreases in volume during the process of milking is a matter of common observation. It has been stated [see Marshall (4) p. 557] that the udder of a cow could not contain the quantity of milk which can sometimes be obtained from it at one milking. No quantitative data are given to back up the statement. In making measurements of the change in volume of the udder of the goat by removal of the milk I have found in some cases a decrease equal to the amount of milk secured, and in other cases a decrease less than the volume of milk drawn. An average of four determinations shows a yield of 339 cc. of milk and a decrease in volume of udder of 323 cc. The method of volume determination, as previously noted, was not exact. The results of one trial were as follows:

Goat No. 5. 5/5/15, a.m.

	cc.	cc.
Volume of udder	1540	
Milked out	105	
Kids nursed	255	
Total milk		360
Volume of udder	1170	
Decrease in volume of udder by milking		370
Injected salt solution at 25 cm. water pressure		480
Volume of udder	1635	
Increase in volume		465
Milked out (1.1 per cent fat)	185	
Milked out one hour later (2.5 per cent fat)	82	
Kids nursed	265	
Total fluid recovered (1¼ hours)		532

The pressure of injection used was probably somewhat greater than that at which the milk existed in the udder. It is noteworthy, however, that in this case the decrease in volume of the udder by milk-

ing and nursing was equal to the volume of milk removed. Also, the internal capacity of the udder after milking and nursing was shown to be considerably greater than the volume of milk yielded.[2]

Change in fat content of milk with progress of milking. Data previously given serve to emphasize the well known fact that as milking progresses the fat content of the milk rises greatly. The effect may be duplicated to a certain extent by allowing milk to pass through a quantitative filter paper. The results of such a test are given in table 6. The goat was first turned on her back and the milk cisterns gently massaged to destroy the results of any tendency for the milk to cream in the cisterns. 25 cc. was then drawn, designated as fore-milk; then the main portion,

TABLE 6

Showing the fat content and size of fat globules in fore-milk and stripping milk in comparison with similar data for the main portion of the milking, mid-milk, and two fractions of a 50 cc. portion of the mid-milk separated by allowing to pass through a filter paper until 20 cc. had filtered

SAMPLE	FAT	AVERAGE DIAMETER OF FAT GLOBULES
	per cent.	*μ*
Fore-milk, 25 cc	4.8	2.2
Strippings, 40 cc	8.3	2.8
Mid-milk, 245 cc	6.0	2.5
Filtrate, 20 cc	5.3	2.3
Non-filtrate, 30 cc	6.6	2.7
Filter control	6.0	
Creaming control	6.0	

245 cc., designated as mid-milk; and finally the last portions, amounting to 40 cc. and designated strippings.

A 50 cc. portion of the mid-milk was placed in a filter and 20 cc. allowed to filter through, requiring about 20 minutes. The portion remaining unfiltered was transferred with the paper to a beaker, the paper gently washed through the milk and removed. This portion is designated non-filtrate. As controls another 50 cc. portion of the mid-milk was treated the same as the above except the unfiltered part

[2] A characteristic reaction of the goats I have worked with is for them to start chewing the cud when milking begins and to continue during the process of milking. Upon the injection of the salt solution in this trial the goat started again to chew her cud and continued as if she were being milked. That is, the passive distension of the gland produced the same reflex, with reference to this particular, as is usually produced during its active contraction during the milking act.

with the paper was added to the filtrate and the paper, after light washing through the milk, removed. This is designated filter control. Still another 50 cc. portion of the mid-milk was allowed to stand in a beaker during the foregoing process and a sample drawn from the surface. This is designated creaming control.

From table 6 it appears that the filter paper retards the passage of the fat globules more than it does the rest of the milk. The fat content and size of the fat globules in the filtrate are decreased. A certain parallelism appears between this effect and that shown in the natural separation of fore-milk and strippings in the removal of milk from the mammary glands. The effect of treating cow's milk (where the fat globules are larger than in goat's milk) as above, is even more marked on the fat content. Thus, in one case a sample of cow's milk containing 7.1 per cent fat showed in the first portion filtered 0.7 per cent fat.

DISCUSSION

It seems highly probable that the remarkable growth of the mammary gland during pregnancy is excited by the presence in the blood of a specific hormone, speaking in terms of current point of view. The evidence of such natural experiments as that of the pygopagous twins, the transplantation experiments of Ribbert, and the foetal extract experiments of Lane-Claypon and Starling, is readily interpreted in such a way. On the other hand it is clear that the hypothetical hormone is not present in large or potent quantities in the blood of the pregnant animal, or else that the mammary gland of the pregnant female is for some reason more susceptible to its influence than the gland of the non-pregnant female; for the transfusion of considerable quantities of blood does not produce positive results. The negative results of Lombroso and Bolaffio with rats united in parabiosis would seem to overthrow the hormone hypothesis. But the authors state that a starved member of such a pair suffered death as quickly as a normal starved control, even though its parabiotic mate was given full feed. That is, there was, apparently, no interchange of circulating food substances. From this it might be well contended that passage of a possible hormone in the blood of the pregnant individual of a pair to the blood of the other, non-pregnant member, was not possible. The fact that lactation may occur in the virgin does not argue against a hormone of pregnancy being *normally* the main factor in the peculiar increased growth activity of the mammary gland during pregnancy. From a teleological point of view

such a hormone, produced by the foetus (or foetal membranes), would be simply an organ for its own post-natal welfare.

While a development of the mammary gland similar to that occuring in pregnancy is not readily produced by the introduction of blood from a pregnant animal into a non-pregnant female, yet such treatment of a lactating animal immediately and temporarily depresses milk secretion. Either inhibition of milk secretion is due to a different hormone than the one causing growth of the gland in pregnancy, or the gland is more sensitive to its influence in the lactating stage. The Lane-Claypon and Starling theory that milk secretion occurs as a result of the removal of this hormone inhibitor, with the removal of the foetus, serves to explain lactation following normal delivery and abortion. The preliminary preparation of the gland, however, seems to be the essential factor for its secretory activity, since lactation ceases normally after a time even though pregnancy does not recur, and, therefore, without the inhibition of any foetal hormone. Further, while lactation is initiated or increased by premature delivery it is not as large in amount as it is following a normal delivery.[3]

The failure of blood transfusion from the actively lactating goat to a feebly lactating one to accelerate the secretion of milk in the latter indicates that lactation is not due to the presence of some particular substance (hormone) in the blood. The fact that one gland may cease lactation before the other also argues against such a factor. The response of the lactating gland to pituitrin is not dependent on the presence of anything in the nature of an activator in the blood; for its non-lactating mate, supplied with the same blood, does not respond. This supports, indirectly, the contention that lactation is not determined by the presence of any exciting substance in the blood stream.

The form of the curves showing the effect of pituitrin on an active, air-inflated mammary gland shows a muscular response. The relatively rapid rise of pressure as expressive of a contraction of the milk passages,

[3] If this point of view is correct it would seem possible that the foetus, by influencing the development and preparation of the gland preceding delivery, might affect the yield of milk by the mother following delivery. Then, the sire of the foetus, by means of the foetus, might influence the milk yield of the mother. In dairy theory and practice, as far as I know, the bull to which a cow is bred is not recognized as a factor in the yield of milk by the cow following the birth of the calf by that mating. Probably the maximum yield of milk is fixed by the genetic endowment of the milk secreting cells of the gland and influences of pregnancy and delivery serve purely a qualitative purpose in initiating activity and have no quantitative effect. The point, however, is worthy of investigation.

followed by the slow decline as expressive of a relaxation is characteristic of the response of smooth muscle. The failure of a second dose to duplicate the effect of the first is not a thing unusual to the action of drugs. Indeed, with pituitrin itself while a first dose causes a rise of blood pressure due to constriction of peripheral vessels, a second dose may not cause a rise of pressure. That the pressure changes in the gland upon injection of pituitrin are not due to changes of blood pressure seems certain from the fact that no change is produced in the non-lactating gland in a late stage of pregnancy, although the gland is at that time highly vascular. That it is not due to a secretory action of the cells is shown by the rapidity of the rise and the return to the initial pressure. A rise of pressure through a secretory action could occur only by the absorbtion of water and its passage into the ducts. If this occurred it is extremely unlikely that resorbtion would commence at once and be carried to a point that would exactly restore the initial pressure. A muscular explanation seems the only one to fit the data. The difference in sensitiveness of the contractile elements of the gland to pituitrin according to the stage of activity finds an analogue in the response of uterine muscle to adrenalin, where the gravid uterus is strongly contracted and the virgin uterus practically unaffected.

The outflow of milk caused by pituitrin is well explained by a muscular action. This seems the more likely as a correct explanation when it is remembered that the act of nursing causes an active increase of pressure in the gland closely resembling the effect of pituitrin. The flow of milk produced by pituitrin when simply placed on the cut surface of the excised gland containing milk shows that its effect is independent of the circulation (except, of course, to bring it in contact with the gland when injected), and gives, also, pretty conclusive evidence that it causes constriction of the very small milk passages of the gland.

Hammond bases his conclusion that pituitrin stimulates the secretory activity of the cells, and has no muscular action on the gland, on the yield of milk obtained by hand milking during a test period of a few hours. Rather positive evidence of a muscular action has been shown above. It must also be clear, from the data given, that any attempt to measure the secretory activity of the gland cells by the amount of milk that may be obtained by hand milking in a test extending over a few hours, is subject to a large error because of variations in the completeness of removal of the milk from the gland. Milk secretion, in the sense of the formation of the milk constituents, is one thing; the ejection of the milk from the gland after it is formed is quite another

thing. The one is probably continuous; the other, certainly discontinuous. And inasmuch as no one has shown any material increase in the milk obtained over a period of days by the use of pituitrin any true secretory action of the drug must be held unproven.

The data given on the flow of milk during milking and nursing show very plainly that there is a reflex constriction of the gland (very similar to that produced by pituitrin) involved; that the removal of the milk from the gland is dependent upon the operation of this reflex; and that the reflex is conditioned. The stimulus which, naturally, excites the reflex must be found in the friction and warmth of the sucking action of the young acting on the cutaneous sense organs of the teat; with, possibly, a further source in the passive dilatation of the sphincter muscles of the nipple by the passage of the milk.

If the mother dog be placed on her back the pups experience no difficulty in getting the full yield of milk, although it must be secured directly against the force of gravity. But if the mother be placed under ether, in a normal position, the pups are unable to secure any milk. Evidently some connection in the reflex arc is broken by the anesthesia, and the indication would seem to be that the central nervous system is involved. The failure of vigorous and continued nursing to secure milk under this condition indicates that the normal removal of the milk is not passive with respect to the activity of the gland. Pituitrin restores the flow, presumably by acting directly on the contractile elements of the gland and producing the same sort of contraction that is ordinarily produced by the nursing reflex.

An artificial imitation of the stimulus afforded by the nursing kid which must have duplicated the natural stimulus very closely, failed to excite the nursing reflex in the absence of the kid; but with the kid present the reflex was called into effective operation by the artificial stimulus as quickly and thoroughly as by the nursing of the kid himself. This may be interpreted to mean that conduction in the reflex arc is dependent upon the psychic condition of the mother.

When the recently delivered cow or mare has been separated from her young some time and her udder is distended with milk, she shows, when brought to her calf or colt, marked symptons of maternal concern, and accompanying this there is often a spurting of milk from the nipples. Apparently, with the gland under this extreme tension, the psychic state produced by the recovery of the young induces a contraction of the gland and an ejection of milk. But in the case of the goat above, with the udder previously drained of milk by hand milking,

recovery of the young, although causing plainly an altered psychic state of the mother, was not sufficient to cause a further flow of milk. But this psychic state in combination with the mechanical and thermal stimuli of nursing permits a reflex more powerful than that excited by the preceding hand milking and calls into play, as it were, a certain reserve strength in the contractile elements of the gland which results in a large flow of milk. In the dog this seems to be the limit of contractile power of the gland, but in the goat pituitrin causes a still further contraction as seen in the ejection of milk. (The statement is based on trials with one goat only.) It is of interest to note the somewhat analogous action of adrenalin on the vascular system. The drug produces a stronger constriction of the blood vessels than can be produced by any reflex nervous excitation; but direct stimulation of the motor nerve to a vessel may cause an equal constriction.

It is difficult to reconcile the seeming dependence of proper nursing or milking on a central nervous mechanism with the results of Eckhard (18) who found no effect on yield of milk in the goat by section of the nerves to the mammary gland; or, with those of Goltz and Ewald (19) who found that removal of the spinal cord in the dog did not interfere with the nursing of her pups.

The determinations of volume change in the udder with milking here reported are hardly extensive or varied enough to be absolute, but so far as they go the indication is that practically the entire quantity of milk obtained at any one time is present as such in the udder at the beginning of milking. Certainly, the capacity of the ducts and alveoli of the udder in the goat is as great as the milk yield at one time. There is, therefore, no *a priori* reason why the milk should not be regarded as being accumulated as such in the udder during the interval between milkings, and the milking operation regarded as a more or less effectual method of stimulating a reflex which causes a contraction of the gland musculature resulting in the ejection of a greater or less part of the milk therein present, according to the effectiveness with which the reflex is excited. It has been claimed that in the heaviest milking cows it is impossible for the udder to contain the quantity of milk that may be secured from it at one time, but that is a matter for determination rather than speculation.

Histologists, as a rule, have been unable to demonstrate any contractile elements in the alveoli of the mammary gland. Positive physiological evidence, however, should outweigh negative histological evidence. Benda (20) has demonstrated contractile elements in the

basement membrane of the alveoli to his satisfaction. The muscular mechanism of the mammary gland seems to be a very important factor in its functioning. It is, indeed, not inconceivable that the activation of the muscular mechanism of the gland may be an important factor in the onset of lactation. Thus, colostrum is secreted by the cells and fills the ducts during pregnancy long before the contractile mechanism is sensitive to pituitrin or to the stimulus of milking. More extensive data than are here available are needed for definiteness, but, at least, it is clear that the contraction of the gland under the influence of pituitrin runs more closely parallel to the appearance of an actual outflow of milk than does the formation of milk (colostrum) by the secreting cells.

The high fat content of strippings milk as compared with fore milk can not be accounted for by a creaming process in the udder for the same phenomenon occurs in the human (Engel (21)). How far the effect noted by simple passing of milk through a filter paper is comparable to the mechanism that causes the result in the mammary gland, is not altogether clear. It is, however, suggestive that the natural process may be partly one of physical retardation of the fat globules in the rapid passage of the milk through the small ducts which must occur in milking. It is generally regarded that the fat of milk is formed within the secreting cells and the globules then extruded through the ruptured end of the cell. It would seem quite possible, also, that under the strain and stress imposed upon the cells by the reflex constriction produced by the milking act this rupture and escape of fat globules from their free ends may be greatly increased and thus cause the higher fat content of the later portions of milk.

SUMMARY AND CONCLUSIONS

Transfusion of blood from a pregnant goat into a lactating one temporarily inhibits milk secretion. Placental and mammary gland extracts from a pregnant cow have an effect similar to the blood. There is some indication of a subsequent accelerating action in the case of the gland extract. Transfusion of blood from a fresh, heavy-milking goat into a low milking one fails to accelerate milk secretion.

Pituitrin has a muscular action on the active mammary gland causing a constriction of the milk ducts and alveoli with a consequent expression of milk. This action holds, also, on the excised gland in the absence of any circulation. The flow of milk produced by pituitrin

is dependent on the amount of milk present in the gland. There is no evidence of any true secretory action. The non-lactating gland, up to a late stage of pregnancy, is not sensitive to pituitrin.

Nursing, milking, and the insertion of a cannula in the teat, excite a reflex contraction of the gland musculature and expression of milk. There is a latent period of 35 to 65 seconds. Milking is a stronger excitant than the cannula; nursing is stronger than milking; [and the direct action of pituitrin (in some cases) is stronger than nursing]. Removal of milk from the gland is dependent upon this reflex, and it may be completely inhibited by anesthesia. The adequate stimulus for the receptor in the reflex arc is the thermal and mechanical effects of nursing; but the strength of the excitation thus aroused is profoundly modified by the psychic state of the mother—especially striking are anesthesia which greatly weakens it, and recovery of the young after separation which greatly strengthens it.

The internal capacity of the mammary gland of the goat is greater than the volume of milk drawn at one time, and the udder shrinks in volume during milking to nearly the same extent as the volume of milk drawn. Practically all the milk drawn is present as such in the gland at the beginning of milking, and is actively ejected by a reflex contraction of the gland musculature under the stimulus of milking.

Passing milk through a filter paper separates it into fractions of different fat content, somewhat similar to fore-milk and strippings. A physical filtration may account, at least in part, for the rise in fat content of the milk with progress in milking.

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